Vol. 49, No. 1, August, 1960

Russian Original Dated January, 1960

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

TRANSLATED FROM RUSSIAN

CONSULTANTS BUREAU, INC.

more collected reports on...

Soviet Pharmaceutical Research

". . .a storehouse of factual references"

SOVIET research in PHARMACEUTICAL CHEMISTRY | 1956

THIS VALUABLE COLLECTION of translations not only gives researchers a more intimate insight into the thinking, methodology, and trends of the Soviet medicinal and pharmaceutical chemists, but it also reports significant contemporary advances in pharmaceutical research.

These 109 selected papers from the 1956 issues of Journal of Applied Chemistry, Bulletin of the Academy of Sciences–Division of Chemical Sciences, Journal of Analytical Chemistry, Colloid Journal, and Journal of General Chemistry will be a valuable addition to all medical and pharmaceutical libraries and should be accessible to all researchers in the field. Sections may be purchased separately as follows:

I Pharmaceutical Chemistry:
Ion Exchange
Miscellaneous
(22 papers) $12.00

II Medicinal Chemistry:
Anti-infective compounds
Steroids and related compounds
Heterocyclic compounds
Miscellaneous
(70 papers) 45.00

III Pharmacognosy:
Alkaloids
Miscellaneous
(17 papers) 10.00

THE COMPLETE COLLECTION $60.00

"At a time when the Western World eyes the achievements of science in the U.S.S.R. with a mixture of admiration and apprehension, it is gratifying to be able to read the English translation of hundreds of Russian articles in the fields of Pharmaceutical Chemistry (Vol. 1), Pharmacognosy (Vol. 2), and Medicinal Chemistry (Vol. 3)... In the application of quantitative physical measurements to problems of pharmacy, the careful recording of data and their evaluation can be termed excellent. American pharmaceutical investigators will have much to learn from these researches (Vol. 1)... Libraries of departments of medicine, chemistry, and pharmacy in universities and the pharmaceutical industry will find it essential to acquire the books, and for some specialists the pertinent volumes may become a storehouse of factual references."–*Alfred Burger in Journal of Chemical Education*

SOVIET PHARMACEUTICAL RESEARCH (1949-55)

I Pharmaceutical Chemistry:
Solubility
Stability
Ion Exchange
Emulsions
Suspensions
Gels
Miscellaneous
(74 papers) ..... $ 95.00

II Pharmacognosy:
Alkaloids
Oils
Glycosides
Miscellaneous
(87 papers) 90.00

III Medicinal Chemistry:
Structure-Activity Relationships
General
(91 papers) 100.00

THE COMPLETE COLLECTION $200.00

NOTE: Individual papers from each collection are available at $7.50 each. Tables of contents sent upon request.

CONSULTANTS BUREAU 227 West 17th Street • New York 11, N. Y.

EDITORIAL BOARD OF BYULLETEN' EKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

V. N. Chernigovskii *(Editor)*
A. Ya. Alymov
S. Ya. Kaplanskii
G. N. Kryzhanovskii *(Secretary)*
L. D. Liozner
M. N. Livanov
I. N. Maiskii
V. A. Negovskii
V. V. Parin *(Acting Editor)*
V. A. Sanotskii
L. L. Shik
A. A. Solov'ev
N. N. Zhukov-Verezhnikov *(Acting Editor)*

BULLETIN OF

EXPERIMENTAL BIOLOGY AND MEDICINE

A translation of BYULLETEN' EKSPERIMENTAL'NOI BIOLOGII I MEDITSINY, a publication of the Academy of Medical Sciences of the USSR

(Russian original dated January, 1960)

Vol. 49, No. 1 August, 1960

CONTENTS

	PAGE	RUSS. PAGE
PHYSIOLOGY		
Summation of Excitations During Unconditioned Reinforcement of a Conditioned Reflex. B. I. Khodorov	3	3
Representation of the Internal Organs in the Cat and the Dog Cerebral and Cerebellar Cortex. III. Representation of the Pudendal Nerves in the Cat Cerebral Cortex. K. M. Kullanda	7	8
Parietal (Contact) Digestion. A. M. Ugolev	10	12
Developmental Changes in the Reflex Control of Osmotic Pressure. L. I. Kurduban and Ya. D. Finkinshtein	14	17
The Part Played by Adrenalin in the Reflex Control of the Thyroid Gland. M. G. Amiragova	17	21
PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY		
Quantitative Measurements of the Sensitivity of the Central Nervous System to Ionizing Radiation. A. B. Tsypin and Yu. G. Grigor'ev	21	26
Effect of Hemisection of Medulla in Puppies and Adult Dogs. E. A. Asratyan and L. S. Goncharova	24	30
Certain Bodily Reactions to Cooling the Brain. N. V. Semenov, G. A. Chetverikova, and T. I. Konstantinova	28	35
The Action of Sera of Healthy Subjects and Anemic Patients on Erythropoiesis. II. The Mechanism of the Erythropoietic Action of Serum. S. Yu. Shekhter	31	39
Functional Changes in the Central Nervous System in Tetanus and the Mode of Action of the Tetanus Toxin. III. The Central and Peripheral Action of the Tetanus Toxin. G. N. Kryzhanovskii	34	42
The Barrier Function of the Lymph Nodes in Infection. N. V. Medunitsyn	40	49
The Effect of Sodium Amytal on the Passage of Sugar from Arterial Blood into Tissues of the Hind Limb and Brain. C. G. Genes and P. M. Charnaya	44	54
BIOCHEMISTRY AND BIOPHYSICS		
The Influence of Methionine and Lipocaic on the Lipid Metabolism of the Liver of Rats Irradiated with X-rays. V. G. Vladimirov	48	59
The Site of the Synthesis of Protein-Bound Iodine in the Thyroid Gland. V. I. Levenson	51	62
The Influence of Sympathetic Denervation on the Content of Glycogen and High-Energy Phosphorus Compounds in the Myocardium. V. A. Govyrin	54	67
The Functional Interaction of Vitamins C, P, and B_1. E. F. Shamrai and T. V. Fetisova	57	70
PHARMACOLOGY		
On the Mechanism of Action of Ganglioplegics on Peripheral Viscero-Visceral Reflexes. V. V. Zakusov and O. V. Ul'yanova	61	75

Annual Subscription $20.00
Single Issue 10.00

Copyright 1960 Consultants Bureau Enterprises, Inc., 227 West 17th St., New York 11, N. Y.
Note: The sale of photostatic copies of any portion of this copyright translation is expressly prohibited by the copyright owners.

Central Antagonism Between Gangliolytic Agents and Neostigmine Methylsulfate. M. L. Tarakhovskii 64 78
Effect of Glutamic Acid on Cholinergic Excitation in Central and Peripheral Synapses. P. E. Dyablova 68 83
MICROBIOLOGY AND IMMUNOLOGY
Changes in the Antigenic Properties of the Blood Serum After Parenteral Injection of Nonprotein Substances into an Animal. A. I. Nikolaev 71 87
Electrophoretic Analysis of the Composition of the Specific Precipitate. Yu. S. Tatarinov 74 91
ONCOLOGY
Investigation of the Action of a Number of Antitumor Preparations on Cultures of Human Tumors. T. G. Khaleeva 77 95
EXPERIMENTAL BIOLOGY
Regenerative Hypertrophy of the Skin in Rats. Yu. K. Man'ko 80 99
The Influence of Inflammation on the Regeneration of Muscle in Experiments with Minced Muscle Tissue. R. P. Zhenevskaya 84 104
The Effect of Irradiation of the Recipient with X-rays on Homoplastic Transplantation of a Peripheral Nerve. Yu. M. Gudzovskaya 89 110
The Effect of Autonomic Stimulators on Cell Division in the Corneal Epithelium. E. S. Tsareva 92 115
MORPHOLOGY AND PATHOMORPHOLOGY
The State of the Lymphatics During Disturbance of the Venous Return from the Limbs in Dogs. A. G. Fedorova 95 118
The Spinal Sensory Component of the Ganglia of the Sympathetic Nervous System. N. K. Landshman 98 122
METHODS
The Technique of Embedding Dry Anatomical and Biological Specimens in Organic Glass (Methyl Methacrylate). A. V. Perelygin and N. V. Krylova 101 126

The National Institutes of Health of the Public Health Service has supported in part the cost of publishing BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE through the purchase of copies for distribution to American medical libraries.

SUMMATION OF EXCITATIONS DURING UNCONDITIONED REINFORCEMENT OF A CONDITIONED REFLEX

B. I. Khodorov

Physiological Laboratory (Deputy – Professor L. L. Shik) A. V. Vishnevskii Institute of Surgery (Director – Active Member AMN SSSR A. A. Vishnevskii) AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 3-7, January, 1960.

Original article submitted July 29, 1959

We have shown previously [5] that, as a rule, an unconditioned defensive motor reflex is increased by application of the conditioned stimulus which it is used to reinforce. The effect is particularly well shown when threshold ("weak") or suprathreshold ("medium") electrical cutaneous stimulation is used for the unconditioned reinforcement. A strong unconditioned reflex shows little or no increase when the conditioned stimulus is applied. From these results we concluded that at the moment of unconditioned reinforcement there is a summation of conditioned and unconditioned excitations.

However, in the experiments referred to, the conditioned reflexes were usually brought about by "strong" or "medium" electrical cutaneous stimulation and a weak threshold or subthreshold stimulus was applied only in certain special cases. This method did not exclude the possibility that the increase of the weak unconditioned reflex by the conditioned reflex might be brought about not only by summation of excitations but also by repeated application of a strong electrical stimulus forming a response pattern (stereotype) in which there was a strong response to the reinforcing stimulus: the weak current used instead of the strong might in this way evoke the stereotype.

In order to test this hypothesis special experiments were carried out in which the strength of the unconditioned reinforcement was changed for long periods. With this arrangement, the results were the same as those obtained previously [5], which confirmed our view of the summation of the conditioned and unconditioned excitations during the combined application of the conditioned and the electrical cutaneous stimuli.

METHOD

The experiments were carried out on two dogs (Rusty and Silva) from March to May, 1958. The Bekhterev-Protopopov method involving the defensive response to electrical stimulation was used. The stimulus consisted of 1-msec square waves applied at a frequency of 70 cps to the right hind leg. Limb movements were recorded using Petropavlovskii knee-caps connected pneumatically to a Marie's capsule, while respiration was recorded using corrugated tubing similarly connected.

At the start of the experiment, in Rusty we developed a stereotype of responses to stimuli applied in the following order: bell, metronome (M-120), differentiation to the metronome (M-60), interrupted light, and bell. All these stimuli except the bell acted for 10 sec, during the last 2 sec of which electrical reinforcement was given. The bell sounded for 30 sec, during which time electrical cutaneous reinforcement was given from the 8th to the 10th and from the 28th to the 30th seconds so that the conditioned reflex showed two maxima, one toward the beginning and one at the end of the period of application of the conditioned stimulus.

At the start of the experiments the thresholds for the reflexes were determined and an appropriate strength of electrical cutaneous reinforcement selected (in the figures, these test are shown at the start of each kymogram). Tests of the isolated action of the unconditioned stimulus were carried out subsequently for the usual set of reflexes.

RESULTS

The initial state of the conditioned reflexes in Rusty is shown in Fig. 1. In this experiment for reinforcement we used a suprathreshold electrical reinforcement of 30 v, and the effect of this stimulus by itself is shown at the start of the trace. It can be seen that during the action of the bell (279) the current caused a far stronger motor reaction than when applied by itself.

The electrical stimulus (13 v) was then again given three times in succession, and in all cases the animal reacted by a very feeble withdrawal of the foot. At the end of the experiment (Fig. 1), a stimulus

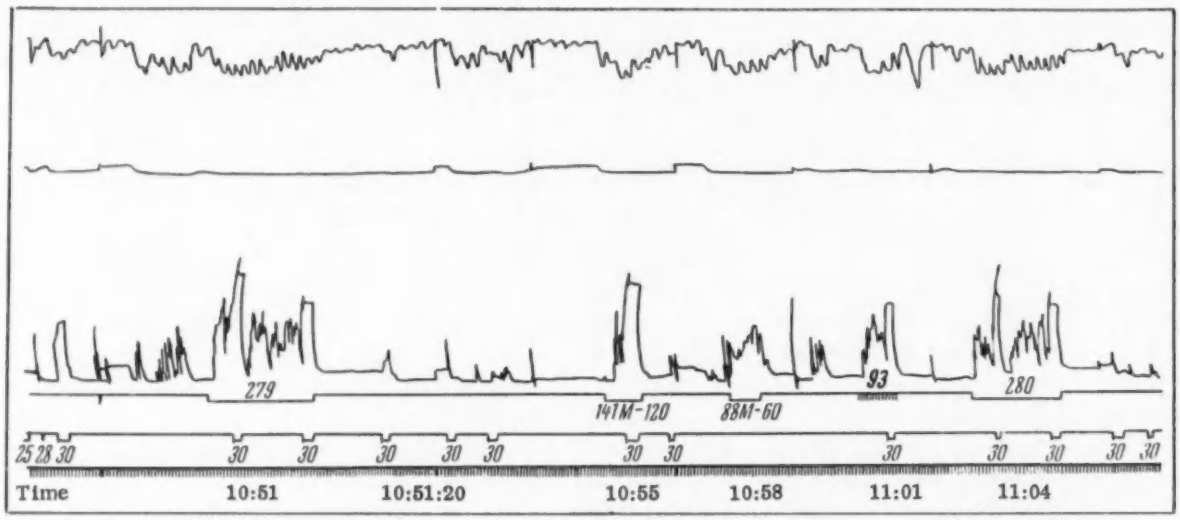


Fig. 1. Motor responses to unconditioned electrical cutaneous stimulus applied either by itself or together with a conditioned stimulus. Experiment on the dog Rusty (No. 105). Initial level of conditioned reflexes. Curves from top to bottom: pneumogram (inspiration downward), movement of the left hind limb, movement of the right (stimulated) limb; conditioned stimulus marker, unconditioned stimulus marker, time marker (...). Numbers on fourth line represent the number (in order) of the conditioned stimulus applied: no letters) bell; M-120) metronome positive; M-60) differentiation; number (bold type) interrupted light. Numbers below fifth line: stimulus voltage in scale divisions of stimulator.

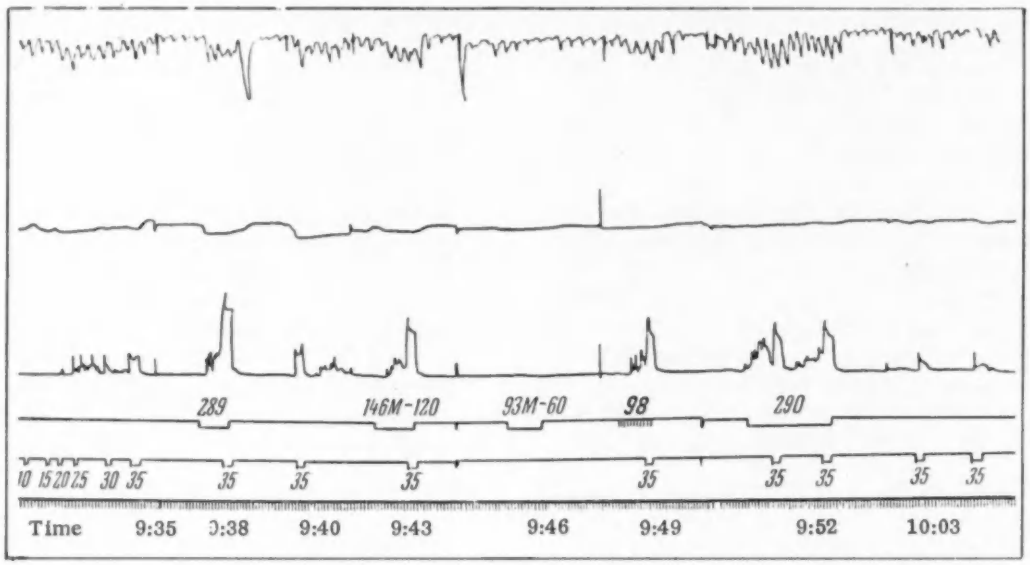


Fig. 2. Summation of weak conditioned and unconditioned reflexes. Continuation of experiments on Rusty (No. 110). Indications as in Fig. 1.

of this strength was almost without effect, as the animal had become adapted to it. Nevertheless, in all tests with the metronome (141 M-120), light (93) and bell (280), the unconditioned stimulus caused a strong motor reaction.

From experiment 109 onward, only a weak electrical cutaneous stimulus was used as reinforcement. A gradual reduction in the strength of the unconditioned reinforcement led to a corresponding weakening of the conditioned reflexes to all stimuli. In spite of this result, the use of weak electrical stimulation together with the conditioned stimulus caused an increased effect in all cases (Fig. 2).

In the succeeding experiments, from No. 114 onward, a stronger unconditioned reinforcement was applied, and this led to the rapid restoration of the conditioned reflexes. However, in this case the application of a stimulus of moderate strength together

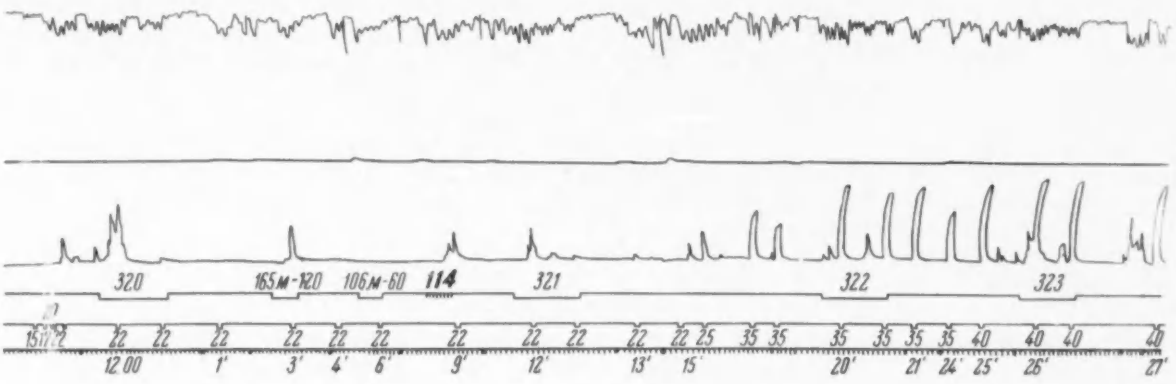


Fig. 3. Change of response with transition from a weak to a strong unconditioned reinforcement. Continuation of experiments on Rusty (No. 123). Indications as in Fig. 1.

with the conditioned stimulus caused an increased effect, while a still stronger unconditioned stimulation caused approximately the same strength of motor response whether given separately or combined with the conditioned stimulus.

Similar results were also obtained in experiments Nos. 116-119.

In the next set of experiments, the results were obtained first, as before, by greatly reducing the strength of the unconditioned reinforcement, and then suddenly increasing it to a high value.

The first tests with the weak reinforcement were used in experiment No. 119, and it was continued from then on. Reduction in the strength of reinforcement in experiments Nos. 120-123 caused a return to the reduced values of the conditioned reflexes. However, when combined with weak or even imperceptible conditioned effects, the unconditioned stimulus regularly evoked a stronger motor response than it did when given alone.

The results of the final experiment of this series are shown in Fig. 3. At the start of the experiment, all conditioned reflexes were reinforced by a weak shock of 22 v. Although after the first few applications, this stimulus changed from being threshold to being subthreshold so that when given alone it caused no overt response, when given together with the conditioned stimuli it continued to cause a definite reflex response (165 M-120, 321). By contrast, when the differentiated metronome stimulus (106 M-60) was used, an electrical cutaneous stimulation of this strength was completely ineffective.

In the same experiment (see Fig. 3) the effect was tried of giving a strong unconditioned reinforcement, for which purpose the voltage was rasied from 22 to 35 v, after which it was given together with the bell 322 at 40 v. It can be seen from the figure that the rapid transition from the weak to the strong unconditioned reinforcement did not destroy the summation of the conditioned and unconditioned excitations (Test 323).

Similar results were obtained in experiments on Silva. Gradual change in the strength of the unconditioned reinforcement led to a corresponding change in the strength of the conditioned reflex. However, during the whole of the period of investigation, the effects of threshold and suprathreshold unconditioned stimulation given together with the conditioned stimulus showed a marked increase, and only in the case of the strong unconditioned reflex was there no change.

Thus, in these experiments the results obtained agreed entirely with those of our previous experiments [5], in which the strength of the electrical cutaneous stimulation was varied only in special tests selected from among the usual combination of stimuli.

The consistency of the results obtained indicates that the increase in the strength of the unconditioned reflex under the influence of the conditioned stimulation does represent a genuine summation of excitations in the defensive center and is not the result of the formation of a set pattern (stereotype) of responses of the animal to the electrical stimulation.

The results of this and of the previous investigation [5] show that the summation of the conditioned and unconditioned reflexes during the combined application of the stimuli is very similar to that occurring in the so-called "united" reflexes [6].

In our experiments, the combined conditioned and unconditioned reflexes produced an effect which in many cases considerably exceeded the arithmetic sum of each taken separately; on average, the value of the reflexes was approximately equal to their sum, and finally the combination of two strong or of a strong and weak reflex produced an effect which was equal to or slightly greater than the value of a single strong reflex response. The explanation may be that the conditioned and unconditioned stimuli converge onto the same cortical cells in the area representing the uncon-

ditioned reflex, onto the same cells of the reticular formation of the brain stem, and finally on the same motorneurones of the spinal cord. For this reason, summation of such convergent stimuli ought to obey the same laws as do excitations in nervous centers, i.e., there ought to be facilitation and occlusion [10].*

The mechanism of interaction of excitation both in the spinal cord, where it was discovered, and also in the subcortical and cortical structures and particularly in the reticular formation has been demonstrated recently by direct electrophysiological means.

It must be realized however that in the interaction of a conditioned and unconditioned reflex there may be complications from many factors associated both with the complexity of the interaction between the different elements of the conditioned reflex arc [1, 2, 7, 8] and with the summation of the excitation in cortical and subcortical cells which may be taking part in some rhythmical activity [9].

In our experiments no cases were found where the conditioned reflex was reduced by application of the conditioned stimulus. Should such effects be found, it would appear to us that they could be caused by the development of a strong orienting reaction at the moment of applying the conditioned stimulus.

Our results as well as those obtained using the feeding method [3, 4] show that the function of a conditioned stimulus is not confined to its signalling action. Besides signalling danger and putting into action at the appropriate time a defensive movement, the conditioned stimulus is also able to increase the strength of the unconditioned response to the direct action of the harmful agent. This effect is of considerable biological importance in cases where the conditioned motor response itself does not get the animal out of danger. The Protopopov method which we have used may be said to reproduce experimentally this situation which may occur naturally.

Thus during the unconditioned reinforcement of a conditioned reflex, the two stimuli combine, and according to their physiological effectiveness they may do so to show either occlusion or facilitation.

SUMMARY

After conditioned motor defensive reflexes had been developed in dogs using Protopopov's method, a comparison was made of the effects produced by unconditioned electrical cutaneous stimuli given either separately or together with conditioned stimulus. The strength of the unconditioned reinforcement ranged from threshold to submaximal values. It was shown that during unconditioned reinforcement there is a summation of the conditioned and unconditioned reflexes, two weak reflexes usually giving an effect which is greater than the sum of the separate values, while two moderate reflexes give a result which is almost equal to the summed effect. A strong and a weak or two strong reflexes give an effect which is equal to or slightly greater than the value of the strong reflex. The results obtained show that in the summation there may be either facilitation or occlusion.

* As in original – Publisher's note.

LITERATURE CITED

[1] É. A. Asratyan, Physiology of the Central Nervous System, [in Russian] (Moscow, 1953), p. 147-197.

[2] I. S. Beritov, Individually Acquired Activity of the Central Nervous System [in Russian] (Tiflis, 1932).

[3] A. O. Dolin, Arkh. biol. nauk 61, 3, 23 (1941); Report of the 8th All Union Congress of Physiology, Biochemistry, and Pharmacology (Moscow, 1935), p. 221.

[4] P. S. Kupalov, Arkh. biol. nauk 61, 3, 23 (1941); Report of the 8th All-Union Congress of Physiology, Biochemistry, and Pharmacology [in Russian] (Moscow, 1955), p. 355.

[5] B. I. Khodorov, Zhurn. vyssh. nerv. deyat. No. 6, 852 (1954).

[6] D. E. Denny-Brown, C. S. Therrington, et al., Proc. Roy. Soc. 1927, ser. B, v. 101, p. 262.

[7] J. M. Delgado, J. Neurophysiol., 1955, v. 18, p. 261.

[8] J. M. R. Delgado, H. E. Rosvold, et al., J. comp. a. physiol. psychol., 1956, v. 49, pp. 373-380.

[9] J. Konorski, Conditioned Reflexes and Neuron Organization, New York, 1948.

REPRESENTATION OF THE INTERNAL ORGANS IN THE CAT AND THE DOG CEREBRAL AND CEREBELLAR CORTEX

III. REPRESENTATION OF THE PUDENDAL NERVES IN THE CAT CEREBRAL CORTEX

K. M. Kullanda

Laboratory of General Physiology (Head – Active Member AMN SSSR V. N. Chernigovskii) Institute of Normal and Pathological Physiology (Director – Active Member AMN SSSR V. N. Chernigovskii) AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 8-12, January, 1960

Original article submitted November 24, 1958

By recording primary responses in the cerebral cortex resulting from stimulation of the central ends of the pelvic nerves we have shown that in the cat the area onto which the nerves project lies within the region in which the hind limbs are represented. The focus of maximal activity (FMA) of the first zone, i.e., the area within which primary responses can be found which have maximal amplitude and minimum latent period lies usually on the gyrus cruciatus posterior, near the central part of the cruciate sulcus. The FMA of the second zone lies on the anterior ectosylvian gyrus in the middle part of the ectosylvian sulcus [2, 3]. It is known however that afferent fibers of many of the internal organs such as the urethra, urinary bladder, and vagina run not only in the pelvic but also in the pudendal nerves. Since the cortical representation of the latter has not yet been worked out, we have attempted to determine it. Our experiments on 24 cats have shown that this nerve also has its area of cortical representation which overlaps that of the pelvic nerves. Nevertheless, their FMA do not coincide, and for each nerve there is a small territory within the common area in which responses from both nerves can be obtained, where the response from one nerve has a maximum value.

METHOD

The experiment was performed as follows: Recordings were made of the response to stimulation of a single nerve, and then without moving the leading-off electrode, recordings were also made on stimulating the other. Next the electrode was transferred to an adjacent portion of cortex and the whole operation again repeated. In studying the representation of the two nerves in parallel in this way, particular attention was paid to maintaining equal time intervals between separate stimuli applied alternately to one nerve and to the other. The time interval chosen was selected empirically so that the reaction resulting from stimulation of one nerve did not fall in the period of the after-effect of the preceding stimulus applied to the other. Usually an interval of at least one minute was left between stimuli.

RESULTS

Figure 1 shows the results obtained in one such experiment. Comparison of the responses recorded from five points on the anterior ectosylvian gyrus shows that stimulation of the pudendal nerves produces a response having a minimum latent period and maximum amplitude when the recording is made from point 2. On stimulating the pelvic nerve, the response with minimum latent period and maximum amplitude is recorded from point 4. Thus points 2 and 4 are the FMA of the two nerves.

The results of this and many other experiments show that in a single preparation the areas of representation of the pudendal nerves are less extensive than those of the pelvic nerves and lie mostly within the field of the latter. Accordingly, the FMA of the pelvic nerve is somewhat more extensive. By comparing the responses obtained from the two nerves it is clear that stimulation of the pelvic nerve causes responses which have a rather greater amplitude and somewhat smaller latent period than those caused by stimulation of the pudendal nerve (see Fig. 1).

This greater activity of the pelvic nerve takes place both in the first and second zones common to it and the pudendal nerve.

Control experiments. In this electrophysiological method, control experiments are of particular importance as helping to determine correctly the reason for any particular electrical response occurring.

We have several times observed that under light anesthesia the application of a single electrical stimulus to the central end of the pelvic or pudendal nerves may cause reflex contraction of the adductor muscles of the thigh. These contractions may occur as short twitches also in the muscles of the base of the pelvis, and when stronger stimuli are used, more distant muscle groups may also contract strongly. It was necessary to determine any possible relationship between these contractions and the electrical recordings which we made.

The control experiments were performed as follows. A bipolar electrode having an interelectrode distance of 4 mm was inserted into the contracting muscle. The potentials from it were displayed on one of the two beams of a cathode-ray oscillograph. The other beam showed the electrical responses in one of the cortical areas of the nerves concerned. Records were made before and after immobilizing the animal. Immobilization was secured by using a Russian preparation named diplacin. It was chosen because when injected intravenously in doses sufficient to cause paralysis it had no noticeable effect on the cortical potentials recorded, as was also found by G. D. Smirnov [4] for the "reactive potentials" of the visual cortex.

Figure 2, traces 1 and 2 shows the results of one such experiment. Trace 1 was obtained before and trace 2 after injecting 0.5 cm^3/kg of a 2% solution of diplacin. Comparison of the two traces shows that the primary response in the cerebral cortex results from stimulation of the afferent fibers of the pelvic nerve and not from the associated reflex contraction of the muscles, because otherwise there would be no response in the complete absence of any muscular contraction (trace 2).

Concerning the action of diplacin, it must be noted that with the dose we used the effect on the electrical responses depended to some extent on the depth of anesthesia. With light anesthesia the injection of diplacin had no effect on the potentials evoked. But if the animal was deeply anesthetized, injection of diplacin reduced the amplitude of the primary response somewhat and increased the latent period (compare traces 1 and 2, Fig. 2). However, increasing the intensity of the stimulus caused a return of the response to the previous amplitude and to the previous value of the latent period.

It remains to describe the effect of the reflex muscular contractions on the electrical cortical response following stimulation of an afferent nerve. Amassyan found that reflex contaction of the lower intercostal muscles caused by stimulating the splanchnic nerve in cats caused a third early wave to occur in the first representational area. The injection of d-tubocurarine eliminated this wave [5]. In the experiment shown in Fig. 2, traces 1-2, injection of diplacin caused some change in the wave form of the response: the second negative wave which occurs in trace 1, is much smaller in trace 2. We never found that diplacin caused any

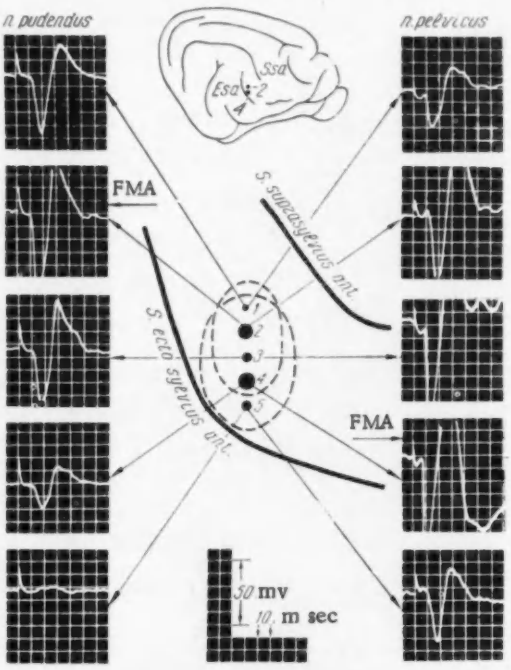


Fig. 1. Comparison of the areas of representation of the pelvic and pudendal nerves in the second contralateral area. Cat No. 162; experiment performed January 18, 1957. Deep anesthesia with a mixture of chloralose and nembutal. Animal immobilized with diplacin. Artificial respiration. Stimulating voltage 7.5 v. Pulse length 0.2 msec; Ssa--S. suprasylvius ant.; Esa--S. ectosylvius ant. Potential in microvolts, time in microseconds.

more definite changes in the shape or size of the different components of the response.

In most experiments, the injection of diplacin caused no noticeable change in the cortical response.

Although muscular contraction caused little effects on the primary response, we were nevertheless careful to immobilize the animal. This was done in order to eliminate completely any artifact, and also because the artificial respiration applied to the paralyzed animal guaranteed a more constant level of oxygenation of the blood than would be obtained during an experiment lasting several hours under normal conditions.

It remains to suggest one further possibility. It might be thought that the responses we recorded occurred through the excitation of other nerves by escape of current. To exclude the possibility we produced a cold block proximal to the stimulating electrodes. When this was done, despite the stimulation of the nerve, no primary responses were obtained. Figure 2 gives the results of one such experiment (traces A-F). Trace A shows the primary response in the first contralateral cortical area due to stimulation of the pelvic nerve. Trace B was obtained two minutes after the onset of cooling the nerve. It can be seen that with the same strength of stimulating cur-

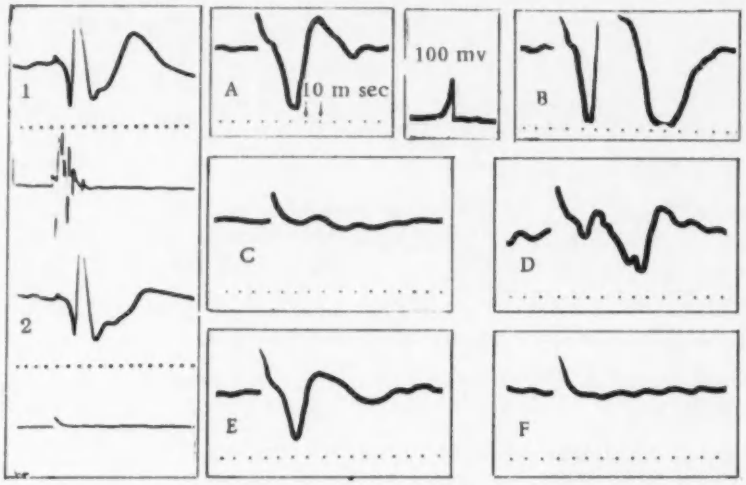


Fig. 2. Traces 1-2. Independence of electrical response from first area of cortical representation of pelvic nerve from reflex contraction of muscles. Cat No. 171; experiment performed January 31, 1957. 1) Before, 2) after injecting diplacin. First and fourth curves) cortical response; second and fifth curves) time marker (10 msec); third and sixth curves) electromyogram of adductor muscles of thigh. Artificial respiration. Chloralose anesthesia. Stimulus strength 5.5 v; duration 0.2 msec. Traces A-F. Cold block and pressure on afferent nerve prevent the development of the cortical response. Cat No. 74; experiment performed October 23, 1955. Deep chloralose anesthesia. Stimulus pulse duration 0.2 msec, amplitude 1.6 v. A, B, C) cortical responses (first area of cortical respresentation of pelvic nerve) developing on stimulation of the cooled pelvic nerve; D, E) record from the same cortical point when the nerve was heated; F) pressure applied to the nerve above the stimulating electrodes—cortical response eliminated.

rent, the amplitudes of the primary response have increased, and a secondary response is also present. The changes in the primary response are evidently due to the cooling of the nerve, which passes through a stage of heightened excitability (N. E. Wedensky, 1901). Trace B obtained four minutes after cooling shows that the cold block in the pelvic nerve prevents the primary response occurring. Trace D was obtained some time after cooling had ceased. Now stimulation of the nerve with a single shock of the previous intensity is once more followed by a cortical response. Ten minutes after cooling had ceased, excitation had returned to the original level, and the cortical response (trace E) shows little difference from that which was recorded before the cold block was applied (see trace A). At the end of the experiment the nerve was crushed proximal to the stimulating electrodes. This caused an irreversible block of all cortical response (see trace F, Fig. 2).

In our opinion, the results obtained show that the cortical responses obtained depend on excitation occurring in the fibers of the nerves stimulated.

SUMMARY

By recording primary responses in two cortical areas of the pudendal nerve, afferent fibers have been shown to run to the cortex of each cerebral hemisphere. The focus of maximal activity (FMA) of the first zone, i.e., the cortical area within which the primary responses occur at maximum amplitude and minimum latent period is usually located on the posterior cruciate gyrus. The FMA of the second zone is located on the anterior ectosylvian gyrus. Both zones lie in the area representing the hindlimb. The cortical areas of the pudendal and pelvic nerves discovered previously by the present author overlap each other widely, though their FMA do not coincide. This signifies that for each of the nerves, within the boundaries of the common area in which primary responses occur, there is a small area in which maximum values are obtained.

LITERATURE CITED

[1] N. E. Wedensky, Selected Works [in Russian] (Leningrad, 1951) 2, p. 509.

[2] K. M. Kullanda, Notes on the Scientific Works of the AMN SSSR for 1955 [in Russian] (Moscow, 1956) p. 114.

[3] K. M. Kullanda, Byull. éksptl. biol. i med. 43, 5, 3 (1957).*

[4] G. D. Smirnov, Report of the Nineteenth International Physiological Congress [in Russian] (Moscow, 1953), p. 189.

[5] V. W. Amassian, J. Neurophysiol., 1951, v. 14, p. 435.

*Original Russian pagination. See C. B. Translation.

PARIETAL (CONTACT) DIGESTION

A. M. Ugolev

Laboratory of General Physiology (Head – Active Member AMN SSSR V. N. Chernigovskii), Institute of Normal and Pathological Physiology (Director – Active Member AMN SSSR V. N. Chernigovskii) AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 12-17, January, 1960

Original article submitted July 30, 1959

For more than 100 years it has been generally supposed that digestion in the higher animals takes place as follows:

The food passes into the cavity of the digestive tract, becomes finely divided and mixed with the secretions of various glands so as to form a chyme. In the chyme, under the influence of various enzymes the food becomes broken down into nonspecific constituent elements capable of being absorbed.

This view, although generally accepted, involves certain serious contradictions. In particular, in spite of great advances in our knowledge of the physiology of digestion and the chemistry of enzymes, until now it has not been possible to reproduce in vitro those rapid breakdown processes which occur in vivo.

In attempting to understand the conditions which govern digestive hydrolysis in the living animal, we have studied the hydrolysis of starch by amylase in the presence of small pieces of intestine. During the course of the work we obtained results which indicated that cavity digestion (throughout the whole mass of chyme) is not the only or even the main mechanism which brings about the breakdown and absorption of food, and that in addition there are other very important and hitherto unknown processes which we have referred to collectively as parietal or contact digestion.

METHOD

We have studied the effect of small portions of living rat small intestine having a surface of serosa of 1-1.5 cm^2 on the hydrolysis of starch by amylase in vitro.

The rate of hydrolysis was determined photometrically in terms of the amount of starch destroyed, using the Smith-Row method and incubating for 30 minutes at 38° in a phosphate buffer at pH 7.5 containing 0.85% NaCl, or in Ringer's solution; the results obtained by both methods were almost identical.

In the first set of experiments we compared the activity of 1 ml of diluted pancreatic amylase, the activity of a piece of small intestine in 1 ml of phosphate buffer, and the activity of 1 ml of amylase to which a piece of small intestine was added during the period of incubation with starch.

In subsequent experiments we used the "trichloracetic" models of the esophagus, stomach, and small and large intestines.

These were prepared as follows: The animal was decapitated and portions of intestine, stomach, etc., rapidly removed and washed several times in physiological saline cooled to 3-5°. The portions were then transferred for 1½-2 hours to a 10% solution of trichloracetic acid (TCA). After being treated for two days with TCA, the intestine was washed in several changes of Ringer at a temperature of approximately 0°.

RESULTS

It can be seen from Fig. 1 that in the presence of a portion of living intestine, the rate of enzymatic hydrolysis of starch increases, so that it becomes considerably greater than the summed effects of the amylase solution and the intestine when incubated separately with the standard amount of starch. This effect cannot be ascribed exclusively to the activity of the living structures of the intestine or to the presence in the gut of particular enzymes, because the pieces of intestine previously killed by treatment with 10% TCA increase the activity of the amylase, although they themselves have no enzymatic activity.

As can be seen from Table 1, the presence of pieces of dead intestine caused a marked increase in enzymatic activity.

The following explanation of the phenomenon may be advanced: The intestine contains substances which pass into solution and increase the action of

amylase, or alternatively the increase in amylolytic activity results from the fact that the intestine exerts an influence on the kinetics of the enzymatic reaction by presenting an active porous surface.

We have shown that the addition of aqueous or saline extracts of small intestine to the amylase cause no appreciable effect on the hydrolysis of starch as is shown by comparing it with a control mixture containing the same amount of enzyme but no intestinal extract.

It can be deduced from these experiments that the increase in amylolytic breakdown of starch caused by adding intestine is not due to the presence in it of substances activating amylase.

If we suppose that under the conditions of our experiments there is an increase in the activity of the amylase in solution, then it would be expected that first incubating the amylase with pieces of intestine would cause an increased hydrolysis of starch when the latter was added after the intestine had been removed (by analogy with the activation of trypsinogen by enterokinase). However, it can be seen from Table 2, that after incubating with pieces of intestine there is never any increase in amylase activity, but in most cases there is some reduction. These results do not accord with the hypothesis that substances activating amylase pass from the intestine into the solution, or that the enzymatic activity of amylase in the solution is increased.

From this table it can be seen that after incubating with amylase, the intestine acquires a considerable enzymatic activity several times greater than the loss of the enzyme in the original amylase solution. The experiments demonstrate that a certain amount of amylase must be adsorbed onto the surface of the intestine in such a way as to increase the activity of the adsorbed enzyme considerably.

It is usually supposed that the epithelium of the small intestine performs the functions both of absorption and secretion. No experimental study has been made of the problem of how the large surface area affects the course of the enzymatic reactions in the

TABLE 1 Effect of TCA Model of the Small Intestine on Amylase Activity

Number of experiment	Amylolytic activity of hydrolyzed starch, %		
	amylase	intestine	amylase- and intestine
1	28	0	46
2	31	0	52
3	19	0	34
4	12	0	20
5	47	0	57

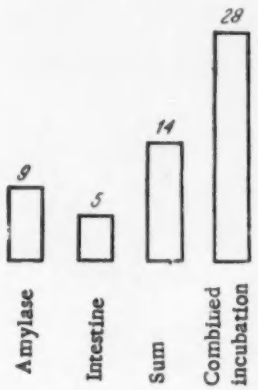


Fig. 1. Effect of a piece of living intestine on the breakdown of starch by amylase. The activity is shown as a percentage of the substrate hydrolyzed. From left to right: activity of a preparation of pancreatic amylase, innate amylolytic activity of the strip of intestine, some of the two activities, activity shown during combined incubation with amylase and intestine.

lumen. Nevertheless it is well known that the rate of enzymatic processes on a surface is very different from that occurring in a fluid medium.

Our results indicate that the large surface of the small intestine performs a further important function – that of reconstituting a living porous adsorbant. Under normal conditions, part of the digestive enzymes remain in solution, and bring about digestion in the lumen, and until now this has been considered the only mechanism responsible for the breakdown of food, while the remaining part of the enzymes are adsorbed on to the surface of the mucosa where they bring about contact or parietal digestion.

Evidently, parietal digestion will be best developed in the small intestine. We have compared the increase in the amylolytic breakdown of starch under the influences of pieces of mucosa from the esophogus, stomach, and small and large intestines (experiments on models previously treated with TCA). It can be seen from Fig. 2, that the enhancing effect is comparatively small for pieces of stomach and large intestine, negligible for fragments of esophogus, but well shown for fragments of small intestine.

This differentiation of the digestive tract does not arise by chance. We have compared the rates of breakdown of soluble (partially hydrolyzed) amylose and insoluble amylose by the enzyme in solution and the enzyme adsorbed onto intestine. It was found that the larger amylose molecules are more intensely attacked by the dissolved enzyme, while the smaller molecules (previously partially hydrolyzed) on the contrary were more rapidly broken down in the parietal layer by the adsorbed enzymes.

Evidently, the very first stages in digestion take place chiefly in the lumen of the digestive tract, and subsequently on the surface of the intestine. This view agreed well with the results given previously which showed that parietal digestion occurs chiefly in the small intestine, i.e., where the greatest amount of hydrolyzed substrate is to be found. The arrangement

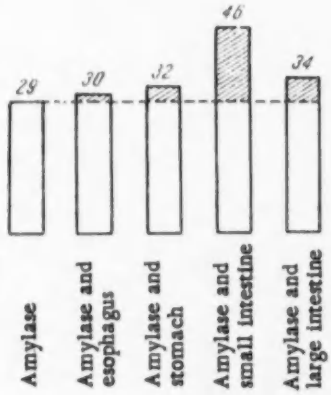


Fig. 2. Effect of equal areas of esophagus, stomach, small and large intestine (treated with TCA) on the activity of pancreatic amylase.

whereby parietal hydrolysis represents the chief supply of absorbable products and takes place directly on the surface of the villi constitutes extremely favorable conditions for absorption. A rough estimate based on our figures shows that the concentration of the starch breakdown products in the immediate neighborhood of the intestinal wall ought to be several times greater than in the chyme. Possibly absorption is not determined by the concentration gradient between chyme and blood, but by that between the parietal layer and the blood.

Because the enzymatic layer on the intestinal surface is related not only to the hydrolysis of food but also to its absorption it ought perhaps to be referred to as the "digestive-transport" layer.

We have found that the adsorbed enzymes are quite firmly fixed to the intestinal surface, and only pass back into solution with difficulty. This indicates that the classical view of the enzymes moving with the chyme along the intestine where they quite soon enter the large intestine to be further broken down or voided to the outside, needs to be refined considerably.

It is usual to suppose that the action of enzymes is limited to the particular portion of chyme into which they have passed when secreted, and that the digestive enzymes pass quite rapidly out of the small intestine together with the chyme, so that at each new act of digestion there ought to be a complete renewal of the enzymes which bring about the breakdown of food. We consider that as a result of adsorption quite a stable enzymatic layer is formed. The result is that the enzymes act on the whole of the food passing past a given section of intestine, and not only on one particular portion of chyme. It is still more important, however, that thanks to the parietal digestion the useful life (if we may call it that) of the digestive enzymes is increased several times. By being adsorbed it is likely that they do not take part merely in one but in several digestive acts.

Probably owing to the presence of two hydrolytic mechanisms (cavity and parietal) and through the interaction of these two mechanisms conditions are established for very effective action on the food. Here, probably the first stages of digestion are brought about chiefly in the lumen (the molecules of the enzymes being oriented with respect to the large portions of substrate), and subsequently hydrolysis takes place on the surface of the villi (the substrate molecules being orientated with respect to the fixed molecules of the enzyme).

Adsorption makes it possible for a particular enzymatic molecule to come into contact with many portions of chyme moving past a given section of intestine. The time of action of the enzymes in the digestive tract is thus increased, and the loss of enzyme is reduced. Finally, the features of parietal digestion as described establish extremely favorable conditions for absorption.

I. P. Pavlov compared the digestive system to a highly perfected chemical factory. The part played in modern chemical engineering by catalysts absorbed on to surfaces helps us to understand the significance of

TABLE 2 Effect on Activity of Incubating a TCA Model of Intestine in an Amylase Solution

Number of experiment	Amylase activity of hydrolyzed starch, %			
	original amylase activity	original intestinal activity	amylase activity after incubating with intestine	intestinal activity after incubating with amylase
1	47	0	47	20
2	47	0	44.5	25.5
3	63	0	56	19
4	59	0	57	20
5	46	0	40	29

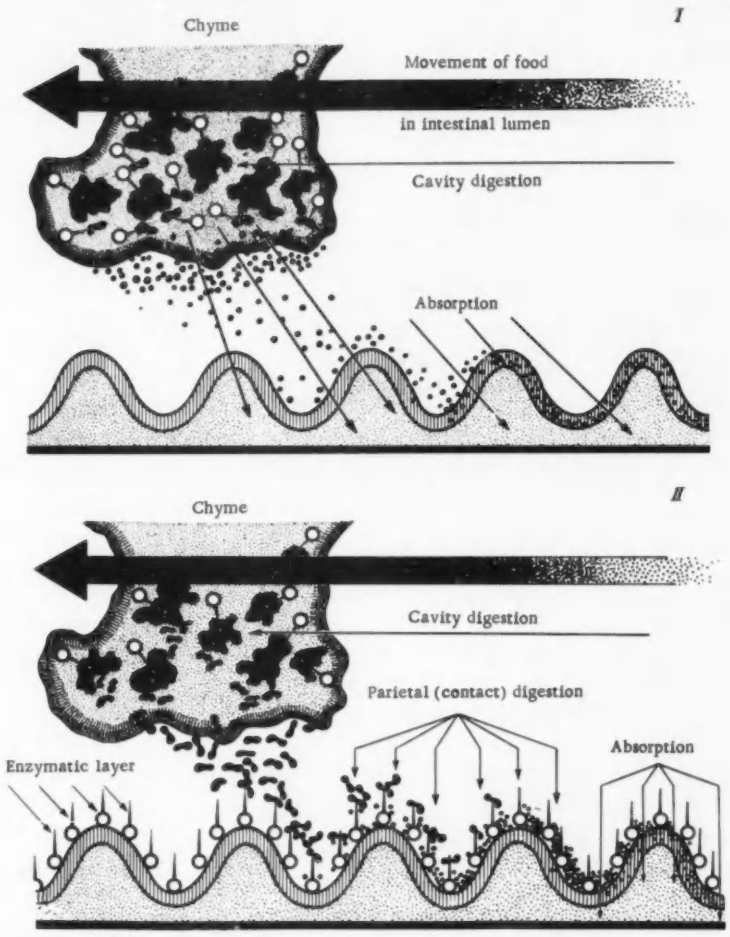


Fig. 3. General scheme of digestion. I) as usually accepted; II) taking into account parietal hydrolysis.

contact digestion in the activity of the living factory which brings about the breakdown and assimilation of food.

The last diagram (Fig. 3) shows the general course of digestion and indicates how cavity and parietal (contact) hydrolyses take place. Above, the usually accepted scheme for digestion is illustrated.

SUMMARY

The evidence presented shows that in higher animals cavity digestion is not the only or even the principal mechanism of breakdown and assimilation of food, but that along with it there is a system of hitherto unknown processes which we here refer to as parietal or contact digestion. The latter occurs under the influence of enzymes adsorbed onto the surface of the mucosa which is concerned in the hydrolysis and absorption of food; a number of specific features of this process are described.

DEVELOPMENTAL CHANGES IN THE REFLEX CONTROL OF OSMOTIC PRESSURE

L. I. Kurduban and Ya. D. Finkinshtein

Department of Normal Physiology (Head – Docent Ya. D. Finkinshtein)
Novosibirsk State Medical Institute (Director – Honored Scientist
Professor G. D. Zalesskii)

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny
Vol. 49, No. 1, pp. 17-20, January, 1960

Original article submitted July 27, 1959

Of the research on the osmoregulatory reflex, the least work has been done on its afferent portion. Verney [5] first describes specific osmoreceptors in the hypothalamic region lying along the course of the internal carotid artery. He thought that only this area was sensitive to changes in blood osmotic pressure. However it was found later that osmoreceptors are by no means confined to the hypothalamic region, but that they are widely distributed throughout the body [1]. A marked reduction in diuresis occurs when hypertonic solutions are injected not only into the carotid artery but also into other vessels, particularly those of the liver.

The object of the present investigation was to study hepatic regulation of osmotic pressure during the early postnatal period: This work included determining the time at which the reflex was formed, studying it in detail, and finding the relation between it and sensitivity to the antidiuretic hormone.

METHOD

The experiments were performed on puppies aged from one day to two months.

A urinary fistula was established under ether anesthesia. Hypertonic solutions were introduced into the splenic vein through a polyethylene canula inserted into it. To do this, the abdominal cavity was opened on the left side, and the spleen removed after tying off all the vessels to it; the canula was now inserted into the splenic vein, and the abdominal cavity sewn up. The canula was taken to the outside, and attached to the skin. The solution introduced through the canula passed effectively directly into the portal vein. Pituitrin was injected into the jugular vein through a second canula.

In puppies up to 10 days old, both operations were performed at the same time, and the experiment begun four hours later. In older animals, the urinary fistula was established first, and the venous canuli introduced after the animal had recovered completely. The experiment was performed one day after the last operation.

The osmotic pressure of the urine was measured cryoscopically using a thermistor. The freezing point was determined to an accuracy of 0.005°.

The puppies were hyperhydrated by injecting 3 to 8% of the body weight of water through the canula. Diuresis was measured at 5 minute intervals and expressed as the amount per square meter of surface area per minute. The hypertonic saline was given in concentrations from 1.5 to 7%. The puppies received 0.25 to 2.5 ml according to age. From 1.5 to 2 I. U. of pituitrin per kilogram weight were given.

RESULTS

A hypertonic solution was injected into the portal vein in 42 puppies aged from 1 to 60 days. The results obtained are shown in the table, from which it can be seen that the number of cases in which there was a reduction in diuresis increased with the age of the puppy.

Whereas in the first three days, no result was obtained in any of the experiments, from the 11th day onwards there was a reduced diuresis in almost all cases.

Besides the antidiuretic action there is also an increased urinary osmotic pressure. It can be seen therefore that the oliguria is not caused by a reduction infiltration but by an increased facultative reabsorption leading to an increase in the concentration of the osmotically active substances in the urine.

The absence of any reaction in the early postnatal period may indicate that the afferent and efferent portions of the osmoregulatory reflex are not yet developed. Evidence on this point is afforded by the reaction obtained from one animal to injections of both hypertonic saline and pituitrin.

It is well known that in the early postnatal period there is practically no response to the antidiuretic hormone [3, 4]. It is also known [3], that pituitrin given in doses even exceeding ten times that which produces a maximal effect in an adult may produce no antidiuretic action in puppies up to ten days old.

In our experiments the reaction to hypertonic saline injections into the portal vein were the same as those obtained with injections of pituitrin. In experiments where a reduced diuresis followed raising the osmotic pressure in the liver, it could be confidently expected that an oliguria would be obtained on injecting pituitrin; also when no oliguria followed saline injections there was no reaction to pituitrin either (Figs. 1 and 2).

In some cases, however, the responses to osmotic stimulation and to the injection of pituitrin were not identical. In two puppies aged 1½ days the saline injection caused no reduction in diuresis, while the pituitrin injection caused a marked though short lasting oliguria.

Measurement of the freezing point of the urine showed that the urinary osmotic pressure was not increased, i.e., that the urine had not become more concentrated. It follows that the oliguria did not result from any specific action of the antidiuretic hormone on the kidney tubules, but was a vascular effect caused by the pituitrin reducing glomerular filtration.

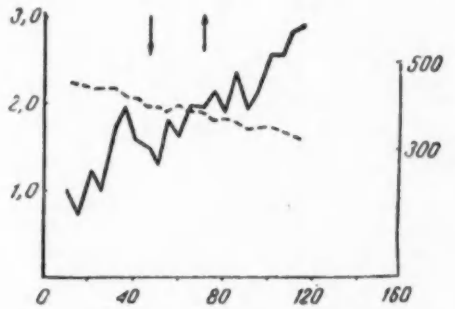


Fig. 1. No reaction to injection of saline (↓) and pituitrin (↑) in a 4-day old puppy. Left ordinate) diuresis in milliliters per square meter per minute; Right ordinate) osmotic pressure of urine in milliosmoles per liter; abscissa) time in minutes; ——— change in diuresis; – – – change in the urinary osmotic pressure.

Effect of Osmotic Stimulus to Liver on Diuresis

Age (in days)	Number of experiments	Response to injected saline	
		oliguria	no effect
1—3	4	0	4
4—7	7	3	4
7—10	10	7	3
10—30	21	20	1
30—60	17	17	0

In five experiments there was a marked response to the injection of hypertonic saline although pituitrin had no effect. Freezing point determination showed that in these cases the osmotic pressure of the urine was increased (Fig. 3).

Since in these animals the kidney did not respond to pituitrin, it must be supposed that the osmoreceptor reflex of the liver influenced the kidneys via the efferent renal nerves and that they were stimulated to reabsorb water [2].

If this was so, it is still not clear why the nervous mechanism does not effect an oliguric response in all cases, when hypertonic saline is given to an animal whose kidney is not yet sensitive to the antidiuretic hormone.

On the basis of the above argument we concluded that the antidiuretic response to hypertonic saline injected into the portal vein develops simultaneously with the sensitivity to pituitrin. The coincidence of both effects shows that the response studied is brought about by the antidiuretic hormone whose liberation stimulates the hepatic osmoreceptor reflex. In most newborn animals whose kidneys are not sensitive to the hormone, there is no antidiuretic response. In a few cases the reflex is brought about without the participation of the hormones, in all probability through the efferent renal nerves.

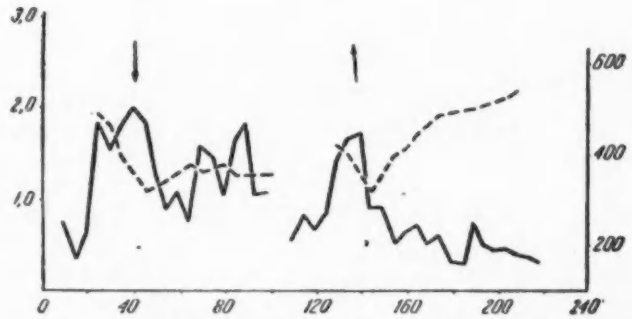


Fig. 2. Oliguria following injection of saline (↓) and pituitrin (↑) in an 8-day puppy. Indications as in Fig. 1.

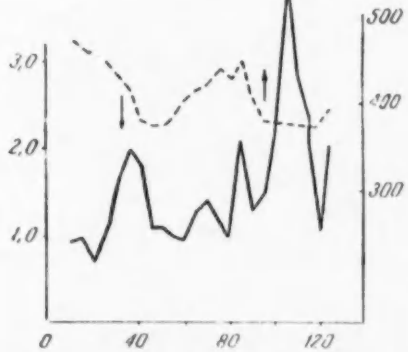


Fig. 3. Response to the injection of saline (↓) and pituitrin (↑) in a 6-day puppy. Indications as in Fig. 1.

The afferent portion of the osmoregulatory reflex becomes established earlier than does its main efferent pathway which is the humoral link in the reflex.

SUMMARY

Experiments on puppies aged from 1 to 60 days showed that the oliguric response to the administration of hypertonic sodium chloride solution into the portal vein occurs at the same time that sensitivity to pituitrin develops. Evidently the reaction is brought about by the antidiuretic hormone whose liberation is stimulated by the hepatic osmoreceptor reflex. In certain cases the reflex is developed without the participation of the hormone, apparently through the action of the efferent renal nerves.

LITERATURE CITED

[1] L. K. Velikanov, Byull. éksptl. biol. i med. 45, 4, 21 (1958).•

[2] A. G. Ginetsinskii and V. F. Vasil'eva, Doklady akad. nauk SSSR 111, 6, 1382 (1956).••

[3] E. A. Nikolenko, in Contributions to Evolutionary Physiology (Moscow-Leningrad, 1957) Vol. 2, p. 151.

[4] H. Heller, J. Physiol., 1944, v. 102, p. 429.

[5] E. B. Verney, Proc. Roy. Soc. London, S. B., 1947, v. 135, p. 25.

•Original Russian pagination. See C. B. Translation.

••See English translation

THE PART PLAYED BY ADRENALIN IN THE REFLEX CONTROL OF THE THYROID GLAND

M. G. Amiragova

Laboratory of General Physiology (Head – Professor V. N. Chernigovskii) Institute of Normal and Pathological Physiology (Director – Active Member AMN SSSR V. N. Chernigovskii) AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten'eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 21-25, January, 1960

Original article submitted March 31, 1959

In previous investigations [2, 3] we demonstrated the relationship between the condition of the central nervous system and the secretory cycle of the thyroid. It was found that during emotional excitement secretion of hormonal iodine from the thyroid was stimulated and its absorption inhibited.

We presumed that these changes were brought about by the liberation of large amounts of adrenalin into the bloodstream. This view was based on the results of Cannon and De la Paz [7] which showed that excitement causes liberation of adrenalin into the blood.

To confirm our hypothesis, we have investigated the effects of intramuscular and intravenous injections of adrenalin on the thyroid secretory cycle.

Several descriptions have been given of the effect of adrenalin on the absorption of radioiodine by the thyroid [5, 6, 8, 9]. This work has shown that adrenalin inhibits the accumulation of radioiodine in the gland. In the investigations referred to, no attention was paid to the effect of adrenalin on thyroid secretory function. We have found no references to any such investigations having been made.

The thyroid secretory cycle consists of two phases – one of hormone formation and the other of its secretion; the authors mentioned above used a method which allowed only one of the two phases to be studied. It is nevertheless evident that any study in which the functional condition of the thyroid is inferred purely from observations made on the uptake of radioiodine necessarily is inadequate.

METHOD

The experiments were carried out on 8 dogs. Intramuscular or intravenous injections of 0.15 mg/kg 1:1000 adrenalin were given. In most of the animals this amount of adrenalin caused a dilatation of the pupils, breathlessness, spasm of the peripheral vessels, vomiting movements, and sometimes actual vomiting. This reaction usually lasted up to 5 minutes, after which the animal settled down again. Since there is considerable variability in the sensitivity to adrenalin, in some cases in order to obtain the reaction described it was necessary to increase the dose of adrenalin up to 0.16-0.17 mg/kg weight.

Radioiodine was given orally, the amount usually being 0.7-0.8 μC/kg weight.

We studied thyroid secretion before injecting adrenalin, and counted the number of impulses over the thyroid gland; the figure obtained was taken as representing the initial radioactivity. After the adrenalin injection, we measured the radioactivity after 15 and after 30 minutes, and then hourly for 3-4 hours, i.e., until it had returned to its original level. Here it must be realized that variations in thyroid radioactivity up to ± 10% were also found under physiological conditions; no attention was paid to any variations of this order occurring in the experiments.

RESULTS

In the first set of experiments we studied the utilization of radioiodine by the thyroid under normal conditions and after an adrenalin injection. Normally, the rate of uptake of radioiodine by the thyroid shows considerable variation, and we therefore decided to study its absorption in the same animal before and after the adrenalin injection. On each ocasion, the animal was used for an experiment only after the whole of the previously injected radioiodine had been eliminated from the thyroid.

It can be seen from the results given in the table that normally after an injection of radioiodine, the amount of it in the thyroid gradually increases; after 4 hours, as a rule the radioactivity of the gland has reached almost twice the values shown during the first hour. The amount absorbed 24 hours after the injection (maximal absorption) was between 21.8 and 35.4% of the total amount injected. These values lie within the normal physiological limits.

After the adrenalin injection, the utilization of radioiodine by the thyroid gradually fell. The effect

Effect of Adrenalin on the Radioactivity of the Thyroid Gland (Figures give the percentage absorption of the total radioiodine injected)

Date of experiment	Before (A) and after (B) injecting adrenalin	Time, in hours					Name of dog
		1	2	3	4	24	
12/XI 1957	A	4,0	5,5	7,6	8,2	21,8	Jack
27/III 1958	B	4,6	5,7	5,6	5,6	13,1	
18/VII 1958	A	8,8	11,2	12,4	14,7	30,6	Coward
21/XI 1958	B	8,4	9,6	9,6	10,0	20,7	
19/IX 1958[1]	A	10,5	12,4	14,2	15,2	35,4	Beetle
1/XII 1958[1]	B	2,6	4,3	5,3	6,3	10,0	
14/I 1958	A	3,0	4,2	5,3	6,5	25,5	Blondy
26/III 1958	B	5,9	6,6	6,9	7,1	15,1	
4/IV 1958	A	3,8	8,8	9,9	—	22,2	Bobtail
23/V 1958	B	3,9	5,8	7,1	8,2	19,2	

[1] Adrenalin injected intravenously.

was usually apparent during the second hour of uptake. The low level of utilization was found not only during the first few hours of uptake, but even after 24 hours. Thus the maximal absorption was considerably less than normal.

This phenomenon was particularly well shown after the simultaneous intravenous injection of radioiodine and adrenalin (see table, experiment 1/XII/1958).

When it is realized that adrenalin is readily oxidized, such a prolonged action at first sight appears unexpected. However it can readily be explained as follows: According to A. M. Utevskii [4], the physiological action of adrenalin does not terminate with its breakdown, and evidently in this case we are concerned not with the action of adrenalin itself but with that of its breakdown products.

Haigh, Reiss, and Reiss [8] have demonstrated a similar effect of adrenalin on the utilization of radioiodine by the thyroid. They found that if adrenalin is injected 30 minutes after giving an iodine injection there is a fall in the absorption curve. This result indicates a reduction in the utilization of radioiodine by the thyroid.

The amount of iodine taken up by the gland is an index of the rate of hormone formation; therefore the studies described lead us to suppose that under the influence of adrenalin the hormonal function of the thyroid is depressed.

However it is still not clear as to whether the values found for the maximal absorption (under the influence of adrenalin) represent a reduction in the rate of hormone formation or whether the effect is due to a more rapid secretion of the hormone from the gland. To answer this question it became necessary to obtain results on the secretory function of the thyroid under the same conditions. The second set of experiments was designed to determine the effect of adrenalin on hormonal liberation.

Once more adrenalin was injected intramuscularly and intravenously. The same result was obtained in each case, and we therefore report only the results for the intravenous injections. It can be seen from Fig. 1 that the action of adrenalin on the hormonal liberation occurs after only 15 minutes and the action is maintained for 1-2 hours. After this time, as a rule, the radioactivity of the gland returns to its initial level.

Only in 17 out of the 55 experiments did we find any noticeable change in secretory function. In the remaining experiments the action of adrenalin was very marked, and in some, the reduction in radioactivity amounted almost to 40% of the original value.

In order to determine the effects of the injection as such and the experimental conditions themselves on the thyroid function, we carried out a series of control experiments in which physiological saline was injected. It was found that the latter had no effect on thyroid function. The change in radioiodine content was not greater than ± 10%.

In assessing the results of the second experiment, it can be seen that adrenalin stimulates liberation of the hormone from the gland into the bloodstream. This in turn shows that the reduced utilization of radioiodine by the thyroid 24 hours after the adrenalin injection is a result not only of the suppression of hormone formation but also of the increased amount liberated.

We also thought it important to find in what way the action of adrenalin in stimulating hormone secretion is brought about. Is the effect, as some people consider, mediated by the hypophysis, or does adrenalin exert an influence on the thyroid directly?

A. L. Botkin and H. Jensen [5] showed that after adrenalin has been injected both the iodine in the blood plasma and that bound to protein is considerably reduced. They consider that adrenalin brings the body into a condition in which the utilization of thyroid hormone by the tissues is increased, and this in turn leads to a loss of hormones from the blood. This loss has the further effect of increasing the secretion of thyrotropic hormone by the hypophysis, which causes

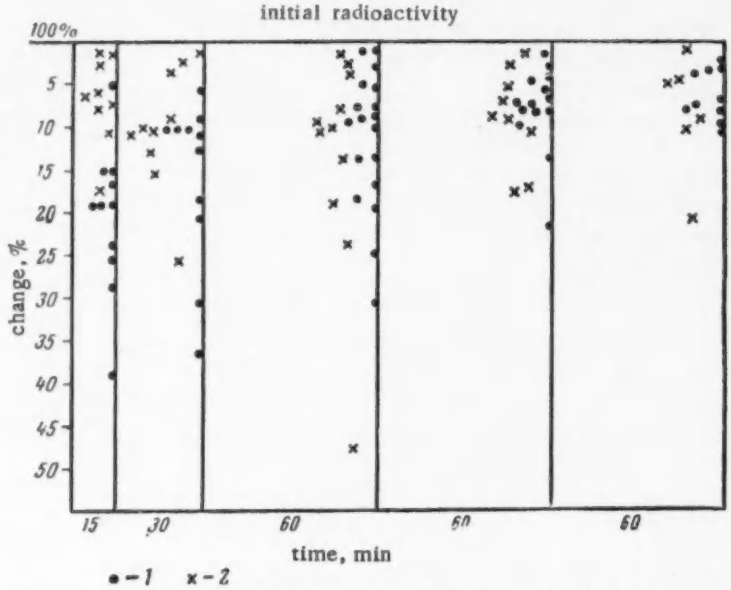


Fig. 1. Effect of adrenalin on the liberation of hormonal iodine from the thyroid (before and after removing the anterior hypophysial lobe). 1) Intact dog; 2) after removal of anterior pituitary lobe.

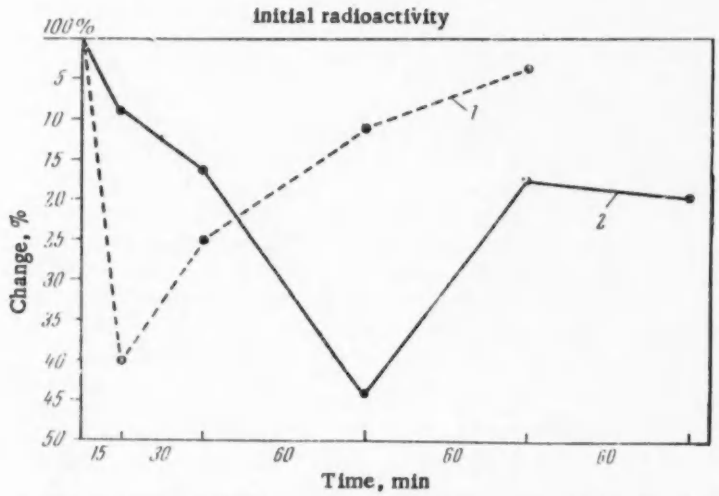


Fig. 2. Effect of adrenalin on the radioiodine content of the thyroid in (1) normal and (2) hypophysectomized dogs.

an increased liberation of thyroid hormone. If this interpretation is true, then it is clear that after removal of the anterior pituitary lobe there ought to be no increase in thyroid secretion.

The experiments carried out on animals after removal of the anterior lobe of the pituitary showed that the injection of adrenalin just as in the case of unoperated animals causes an increased secretion of thyroid (see Fig. 1).

A marked feature of the operated animals was that the radioactivity of the gland after the intravenous adrenalin injection fell not after 15 minutes, but after 40 minutes or even later. Figure 2 shows the results of one of these experiments. The delay in the reaction is understandable when it is realized that hypophysectomy causes a marked reduction in the functional activity of the thyroid.

The response of the thyroid gland to adrenalin after removal of the hypophysis shows that if adrenalin acts on the thyroid as described by A. L. Botkin and H. Jensen, then it must act in some other way also. It therefore seems logical to admit the existence of another mechanism. It is possible that adrenalin acts indirectly on the thyroid. B. V. Aleshin [1] showed

the effect of adrenalin on thyroid tissue in vitro. By incubating pieces of thyroid in a Warburg apparatus to which adrenalin had been added, they observed that the adrenalin had a stimulating effect.

Our experiments have shown that adrenalin changes the functional condition of the thyroid. These changes are similar to those which we found previously to occur during emotional excitement. This fact gives reason to suppose that during excitation adrenalin entering the bloodstream may be one of the mediators of the influence exerted by the central nervous system on the thyroid gland.

Under normal physiological conditions increase in hormone formation and increase in the rate of liberation take place together; similarly, when hormone formation is slowed down, there is also a reduction in the amount liberated. Under conditions when the adrenalin content of the body rises, besides inhibition of hormone formation by the thyroid there is also stimulation of the liberation of the hormone into the bloodstream. The results obtained are important because they enable us to envisage the possibility that the formation and the liberation of the hormone may be influenced in opposite directions; also they confirm that adrenalin stimulates thyroid function.

Previous investigators [5, 6, 8, 9, 10] in studying thyroid function purely in terms of absorption, necessarily came to the opposite conclusion because, as our investigations have shown, adrenalin inhibits formation of the hormone. We have supposed that the reduction in the hormone formation which occurs together with an increase in its rate of liberation represents a "physiological measure," but that the true function of the thyroid under these conditions must be evaluated in terms of its liberation into the blood. It is known that the deciding factor in the activity of any gland including the thyroid is the amount of hormone passing into the bloodstream; it is in this way that the participation of the endocrine glands in the adaptive reaction of the body is determined and their role in the general process of regulation decided.

SUMMARY

A study was made of the part played by adrenalin in the transmission of central nervous influences through the thyroid gland.

Injections of adrenalin decreased the absorption of radioactive iodine by the thyroid and stimulated the liberation of the hormone from it. It had been shown previously that similar effects occur during emotional excitement.

From the effect of adrenalin on the secretory cycle of the thyroid it was concluded that adrenalin is one of the mediators of central nervous control of the thyroid.

Adrenalin may also act on the thyroid without participation of the hypophysis.

LITERATURE CITED

[1] B. V. Aleshin, Development of Pathogenesis of Goiter [in Russian] (Kiev, 1954).

[2] M. G. Amiragova, Fiziol. zhurn. SSSR 43, 1, 65 (1957).

[3] M. G. Amiragova, Byull. ėksptl. biol. i med. 45, 2, 7 (1958).*

[4] A. M. Utevskii, Problems of Endocrinology and Hormone Therapy [in Russian].

[5] A. L. Botkin and H. Jensen, Endocrinology, 1952, v. 50, pp. 68-72.

[6] K. Brown-Grant and J. G. Gibson, J. Physiol., 1956, v. 131, p. 85.

[7] W. Cannon and D. De la Paz, Am. J. Physiol., 1911, v. 28, p. 64.

[8] C. P. Haigh, M. Reiss, and J. M. Reiss, J. Endocrinol., 1954, v. 10, pp. 273-283.

[9] L. J. Soffer, J. L. Gabrilove, and J. W. Jailer, Proc. Soc. Exper. Biol. and Med. 1949, v. 71, p. 117.

[10] F. Verzar, Bull. Schweiz. Akad. med. Wiss., 1953, Bd. 9, S. 121.

*Original Russian pagination. See C. B. Translation.

QUANTITATIVE MEASUREMENTS OF THE SENSITIVITY OF THE CENTRAL NERVOUS SYSTEM TO IONIZING RADIATION

A. B. Tsypin and Yu. G. Grigor'ev

(Scientific Director – Professor M. N. Livanov)

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 26-30, January, 1960

Original article submitted May 30, 1959

One of the most fundamental and pressing problems concerning the biological effects of radiation is to determine the sensitivity to radiation and the conditions which determine it. Nevertheless we are still a long way from having found the answers. Although it is admitted, for instance, that the nervous system responds to comparatively small doses of ionizing radiation, until now little work has been done on the quantitative aspect of this response.

Detailed quantitative work has been carried out on nervous and muscular tissue using electrical stimulation. It has been shown that the strength of the current and its duration are the two parameters which determine excitability. From the facts and direct observations of Gorveg, Weiss, and Lapique the relationship between the value of the threshold voltage and its time of action has been determined. The mathematical relationship between the two has been shown by the so-called strength – duration curve. This curve shows that for each value of the current a corresponding minimal duration of its action on the tissue is required in order that an excitation may be produced.

Following the suggestion of M. N. Livanov, we decided to find to what extent the laws governing electrical stimulation applied also to the action of ionizing radiation on the nervous system.

Until the present time, studies of radiation sensitivity have as a rule been conducted on the basis of determining some end effect such as for instance the period elapsing before a batch of animals died. This approach obviously does not allow the sensitivity to radiation of any part of the body to be determined, i.e., by this means it is not possible to find the threshold value of the radiation which will evoke a response from one particular system.

As an index of the sensitivity of the central nervous system, we used the earliest responses which could be shown on the electroencephalogram. The advantage of this method is that the earlier responses are recorded during the period of irradiation, i.e., during the period in which radiation is being absorbed; this arrangement made it possible to determine the threshold dose.

METHOD

The experiments were carried out on 200 sexually mature chinchilla rabbits weighing 2.5-3 kg. Gamma irradiation was used in order to avoid the possibility of any additional effects arising from electromagnetic fields such as occur when working with x-ray apparatus.* As a source of gamma irradiation we used the original ÉGO-2 and OKFO-1 instruments; this made it possible to expose the animals to a general irradiation of different doses. Among others, we carried out the following experiments: (1) 15 rabbits, dose 7.5 r/sec; (2) 15 rabbits, 5 r/sec; (3) 34 rabbits, 2.5 r/sec; (4) 34 rabbits, 1 r/sec; (5) 34 rabbits, 0.35 r/sec; (6) 34 rabbits, 0.13 r/sec; (7) 34 rabbits 0.013 r/sec. All the animals were irradiated for 5 minutes during which time the electroencephalogram was recorded.

In experiments (1) to (4) the irradiation was absolutely evenly applied, as the cobalt sources were arranged in a ring around the animal. In experiments (5) to (7) the sources were arranged above the animal so that there was a dosage gradient between the upper and lower parts of the body.

The sources were taken from store and transferred to the operating position by mechanical means. Any possible noise effect associated with the operation of the apparatus was excluded by control experiments. In 95% of the animals used, hearing was destroyed by an operation. Also, we carried out experiments in which the Co^{60} was replaced by pieces of metal of the same size and weight. Under these conditions, when all conditions were the same except that there was no radiation actually delivered, no reaction occurred.

As we have pointed out, the index of sensitivity of the nervous system to radiation was taken as the change in the electrical activity of the brain. Poten-

* We gave a report of the biological effect of electromagentic fields developed during the use of x-ray apparatus at the All-Union Conference on Electrophysiology. Report of the All-Union Conference of Electrophysiology (Moscow, 1958).

Time of Development and Extent of Response in Relation to Dose

Number of experiment	Dose rate r/sec	Number of animals in experiment	Onset of response		Extent of response		No response during period of observation
			during first second	during remaining period	Actual numbers	in %	
1	7,5	15	15	—	15	100	—
2	5	15	15	—	15	100	—
3	2,5	34	32	1	33	97	1
4	1	34	21	11	32	94	2
5	0,35	34	17	14	31	91	3
6	0,13	34	1	23	24	74	10
7	0,013	34	—	16	16	47	18

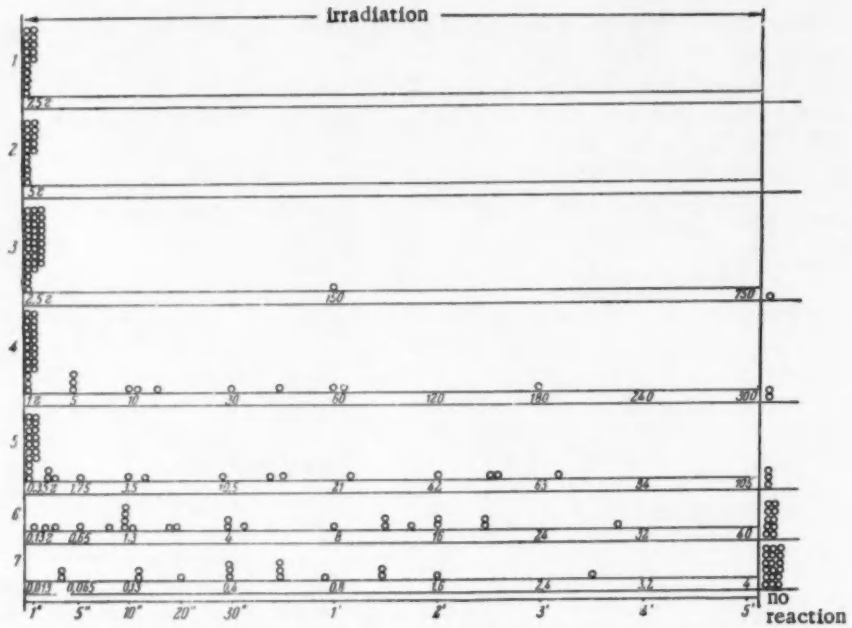


Fig. 1. Time of response occurring during a 5-minute irradiation at different intensities (reaction of each rabbit during irradiation shown by a circle.) Time along abscissa measured from onset of radiation, the number of the experiment is shown on the ordinate as well as the corresponding dose applied.

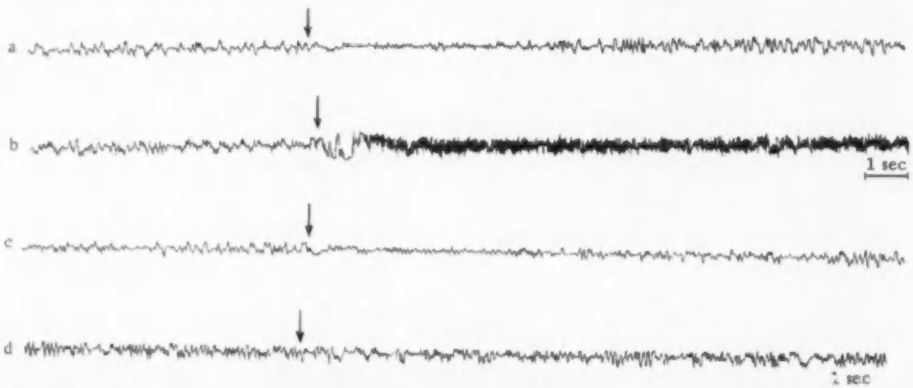


Fig. 2. Type of early change in electrical activity of cerebral cortex of rabbit brain during irradiation (4).

tials were recorded from the parietal region of the cerebral cortex by a monopolar electrode, an indifferent electrode being applied to the skull bone in the midline at the junction of the frontal and nasal bones. The potentials were amplified by a four-stage AC-coupled amplifier. A 100-μv signal applied to the amplifier input gave a deviation of the penwriter of 0.7-0.8 cm. The recording apparatus had a frequency response extending up to 70 cps.

RESULTS

It can be seen from Fig. 1 that in the first, second, and third experiments the reaction occurred in almost all the rabbits at the very onset of the radiation; it was not possible to record the start of the radiation more accurately than to half a second.

The response of the central nervous system was shown as the change in the electrical activity of the cerebral cortex. These changes appeared as frequency shifts and variation in amplitude. These are illustrated in Fig. 2.

Most often, there was a depressor response (see Fig. 2a), and this occurred independently of whether the radiation was given during a period of intense or of weak electrical activity. In some rabbits both the amplitude of the oscillations and the frequency were increased (see Fig. 2b); occasionally the effect of the radiation was to cause a slow regular rhythm to appear (see Fig. 2c). It must be noted that although most of the responses lasted as a rule for not more than 3-12 sec, the frequency change was sometimes maintained for longer, sometimes for the whole period of irradiation.

The reactions described were observed in all the animals in all the first three experiments, and they originated in the first second of the irradiation and were very marked. Two rabbits were exceptions: In one, the response occurred at the 60th second, and in the other no changes were observed for the whole of the 5 minute irradiation period.

In the fourth set of experiments where the power of the radiation was reduced to 1 r/sec, the response occurred in 94% of the animals. However, the time of occurrence and thenature of the response were somewhat different. In 11 of the rabbits, the change did not occur during the first second of irradiation, but later (see Fig. 1). In many cases the reactions were weaker and shorter lasting than previously (see Fig. 2d).

This same difference became accentuated as the dose was further reduced in successive experiments, as can be seen from Fig. 1.

The mean value of the time of onset of the reaction was calculated for each experiment, and the results plotted as a curve in Fig. 3.†

It can be seen from Fig. 3 that the sensitivity of the nervous system to irradiation has the same form as does the sensitivity to electrical stimulation. The curve approximates a hyperbola.‡

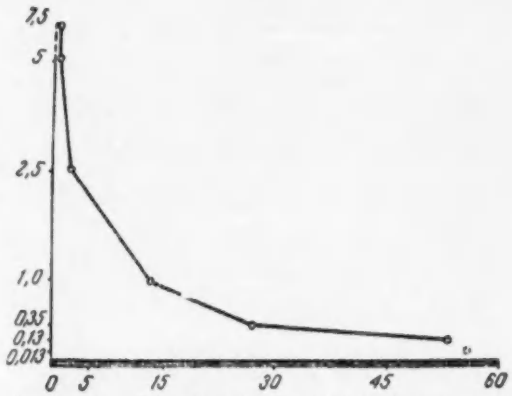


Fig. 3. Curve showing the sensitivity of the central nervous system to irradiation. Dosage is plotted as ordinate (in r sec), time of onset of the response is plotted as abscissa (in seconds).

It is not possible to determine how closely the left portion of the curve approaches the ordinate because it was not possible to determine the onset of the radiation more accurately than to 0.5 sec. As far as the right-hand part of the curve is concerned, there must be some minimal value of radiation which is capable of producing a response when operating for an indefinitely long period. By analogy with the electrical stimulus this value of the dose may be defined as the threshold.

By use of this curve it is possible to evaluate the sensitivity of the different excitable systems in different species, and to demonstrate more convincingly than otherwise would be possible the effect of individual variations. It can be seen from Fig. 1 that as the dose is increased, individual variations in sensitivity cause less alteration in the response to the irradiation.

The experiments we have performed have enabled us to obtain results giving a quantitative indication of the sensitivity of the central nervous system to ionizing radiation and to establish the relationship between excitable systems and radiant action, and also to reveal individual differences in sensitivity.

SUMMARY

This work deals with the quantitative characteristic of the sensitivity of the nervous system to ionizing radiation. The early reaction of the nervous system as revealed by the electroencephalogram was used as an index of sensitivity. The results show the relationship between the time of onset of the reaction and the dose rate. There was a general regularity in the response of the excitable systems to radiation.

† Experiments in which there was no reaction of the central nervous system during the whole of the 5-minute period were not included in calculating the mean values.

‡ The point on the curve referring to experiments with a dosage of 0.013 r/sec cannot be established with certainty by the mathematical treatment used because in 47% of the animals there was no response during the whole of the 5-minute period.

EFFECTS OF HEMISECTION OF MEDULLA IN PUPPIES AND ADULT DOGS

É. A. Asratyan and L. S. Goncharova

Physiological Laboratory of the AN SSSR (Director – Corr. Member AN SSSR É. A. Asratyan), Moscow

(Presented by Active Member AN SSSR V. V. Parin)

Translated from Byulleten' eksperimental'noi biologii i meditsiny
Vol. 49, No. 1, pp. 30-34, January, 1960

Original article submitted September 7, 1959

In considering compensation of function, the after-effects of damage to the medulla are of great practical interest, and one of the foremost problems is that of how to maintain an experimental animal alive for a long time after inflicting different kinds of damage. It is known that the most serious aftereffects are those following the damage to this region, and the outcome is frequently lethal on account of involement of the respiratory and cardiovascular systems [1, 3, 4, 11]. The surgical and neurophysiological aspects of cases where there has been recovery from circumscribed damage to medullary structures have sometimes been reported [4, 13]. In the last few years in our laboratory we have made a study of the aftereffects of damage to this region. In dogs, B. D. Stefanksov [12], and B. D. Stefanksov and V. N. Drozdova have studied the effect of permanent destruction of the dorsal motor nucleus of the vagus.

The object of the present investigation has been to study methods of preserving life for a considerable period after hemisection of the medulla, and also to determine the type of disturbances and the recovery process which occur.

METHOD

A lateral hemisection of the upper third of the medulla was performed in three adult dogs and in thirteen puppies aged 4-8 months. In four of the puppies, the cerebral cortex of one side was removed after the limit of functional restoration had been reached.

Measurements were made of respiration, muscle tone of the limbs, and of the skin temperature of the external ear and limbs. Still photographs and motion picture films were also made.

RESULTS

In three of the adult dogs, a lateral hemisection of the medulla caused breathing to stop immediately. Although artificial respiration applied for 1½-2 hours restored normal breathing the animal died after 12-15 hours from bulbar paralysis and pulmonary edema. On puppies, the result of the operation was different. Of the 13 operated puppies, five died on the first day after the operation; four died on the fourth day, and four survived and were maintained under observation for a long time. In eight, respiration stopped at the time the hemisection was made, and in five the breathing became more rapid. Normal breathing was restored by artificial respiration applied for from 10-15 minutes to 1-1½ hours. As a rule, after the operation, respiration was unilateral and confined to the operated side (Fig. 1a). Bilateral pulmonary respiration was restored very gradually over a period of months. For a long time there was a difference both in frequency and amplitude of the respiratory movements on the two sides of the chest (see Fig. 1b and c). In most puppies there was a well marked Claude Bernard-Horner syndrome (Fig. 2): the eyeball was sunken, the orbital fissure and pupil were narrowed (sympathetic ophthalmoplegia). There was also a paresis or paralysis of the left hypoglossal nerve. In some cases there was a vestibular disturbance shown by a nystagmus which consisted of a rotation of the eye round the longitudinal body axis. Finally, in almost all the puppies the head was rotated so that the left ear was lowered.

It must be noted that unilateral hemisection of the medulla in puppies caused a bilateral disturbance of standing and locomotion. For the first week after the operation, all the animals lay on the side opposite to that on which the operation was performed, and were unable to change their body position. Gradually they learned to use their limbs, and were then able to sit or stand by resting one side against a wall. Muscle tone in the limbs was reduced, and there was also a reduced resistance to passive movement. Not until the 20-23rd day after the operation did the animals begin to walk, and their progress was then unsteady. After 2-3 months, standing and walking became almost normal. After 2-3 months changes in the skin temperature of the external ear and limbs, and also the thresholds of excitability of the flexor reflexes were not well shown.

Thus, the respiratory and locomotor disturbances following unilateral hemisection were compensated after a sufficient time, while the Claude Bernard-Horner

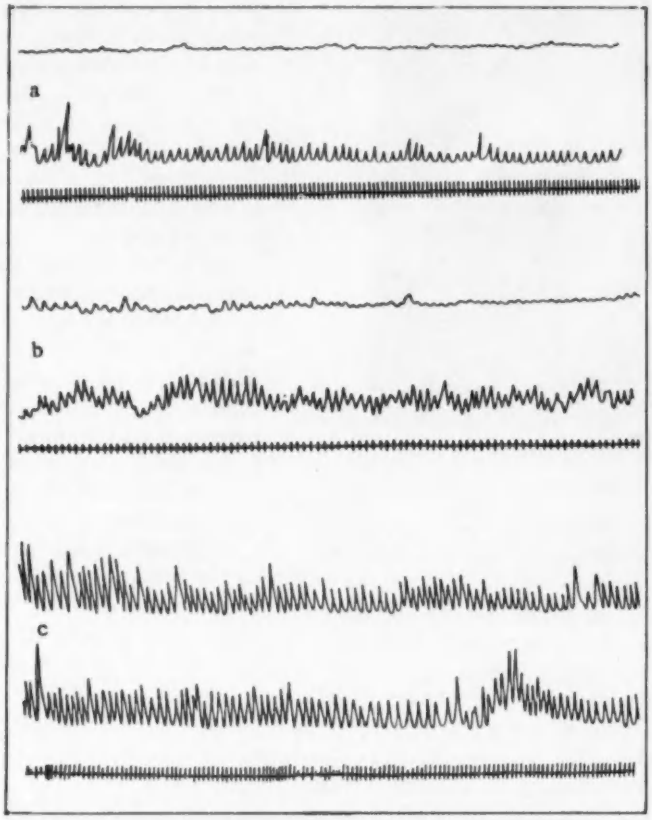


Fig. 1. Change of respiratory movement in the puppy Chita after left medullary hemisection. a) On the 12th day, b) on the 21st day, and c) on the 92nd day after the operation. Uppermost line--respiratory movements of the right side of the thorax; middle line--respiratory movements of the left half of the thorax; lower line--time marker (1 sec).

syndrome and XIIth nerve damage showed no improvement.

To determine the extent to which the cerebral cortex was involved in this compensation, cortical extirpations were made. The cortex of one hemisphere was removed in four of the puppies, in two the hemisphere removed was that on the operated side and in two it was the opposite hemisphere. The latter two puppies died on the third day after the operation from hemorrhage. In two others, removal of the left hemisphere caused recurrent motor disturbances. The animals attempted to rise at the end of the first week, but their movements were uncertain, and the foot was often turned onto the dorsal surface. At first they could stand and walk only with support; by the 21st-23rd day they could run or walk rapidly, but the limb movements were poorly coordinated, and the animals frequently deviated to the side of the operated cortex ("circus" movements).

Removal of the homolateral cortex caused a mild but prolonged respiratory disturbance. After the operation, pulmonary respiration continued to be bilateral, but was asynchronous: On the right the frequency was 30 and on the left 42 per minute, i.e., the rates were the same as were found to occur immediately after the left hemisection of the medulla (Fig. 3).

In these two animals, when no further improvement in standing or in locomotion occurred the second half of the cerebral cortex was removed. This operation caused permanent and irreversible disturbances of stance and locomotion. The animals were unable to stand at all, and made no attempt to rise onto their feet. Periodically there were motor spasms in which the limbs made stepping movements and there was a rotation along the longitudinal body axis. Respiration was bilateral and synchronous, but very variable and the frequency was greatly increased after even short periods of movement. Just as after unilateral decortication, permanent disturbance of the sympathetic enervation to the eye and cranial nerves was maintained.

Histological studies of the brain carried out by F. A. Brazovskaya on three puppies showed that in all cases the upper third of the medulla had been cut through halfway.

Fig. 2. Chita on the 16th day after left medullary hemisection.

The fact that in puppies as opposed to adult dogs life can be preserved after lateral medullary hemisection may evidently be explained as being due to the fact that structural and functional specialization and localization of function in the central nervous system has not been completed. On this account the nervous system of the puppy is more plastic and better able to restore the impaired functions. Evidence obtained earlier by our co-workers [7, 8] and by others [2, 6, 14] confirm that hemisection of the spinal cord carried out during the first months of life cause less marked and less prolonged functional disturbance than does the same operation in adult dogs.

Unilateral respiratory disturbance occurring after lateral medullary hemisection may perhaps be explained as being due to the fact that the connections of the bulbar with the spinal respiratory centers are crossed. The question as to where the crossing takes place has not yet been decided. It has been reported [17] that the spinal projections of the bulbar respiratory structures pass along the anterior and anterolateral fasciculi of the spinal cord. The investigations of Rossi and Brodal [16] on cats have shown that fibres from the cerebral cortex to the reticular formation of the medulla run in the pyramidal tracts. From our results it would appear that the tracts leading from the bulbar structures of the respiratory center to the corresponding spinal centers cross at the same level as do the pyramids. The result of hemisection is therefore to paralyze respiratory movements of the opposite side of the thorax. It may be that pathways crossing, as Pill [15] supposes, above the bulbar respiratory center also play an important part.

Disturbance of stance and locomotion may easily be explained as being due to damage to the extrapyramidal, cerebellar, and other pathways. By comparing the aftereffects of lateral spinal section and lateral medullary hemisection in puppies we can observe a difference in the extent and duration of the impaired supporting and locomotor functions. In the first case, restoration of function occurs much more rapidly and completely. It may be that this is because the medulla even at this age shows greater localization and specialization than the spinal cord, and is of greater importance in controlling these functions. However the problem requires further investigation.

Permanent functional disturbance of the cranial nerves results from the destruction of their nuclei. Sympathetic ophthalmoplegia following lateral medullary hemisection evidently results from damage to the bulbar sympathetic pathways and the results agree with reports [5, 10] that in bulbar paralysis there is damage to the sympathetic supply to the eye.

SUMMARY

The result of lateral hemisection of medulla oblongata was studied on puppies and adult dogs. It was found possible to keep the operated puppies alive, whereas the adult dogs died within 24 hours of the operation. Lateral hemisection of the medulla oblongata led to the development of unilateral reversible disturbances of pulmonary respiration and of locomotion; there was a permanent functional impairment of the cranial nerves and of the sympathetic innervation of the eye (Bernard-Horner syndrome).

Removal of one cerebral hemisphere caused a return of the motor disturbances.

LITERATURE CITED

[1] I. S. Babchin, Collected Scientific Works in Honor of A. L. Polenov, [in Russian] (Leningrad, 1941) p.76.

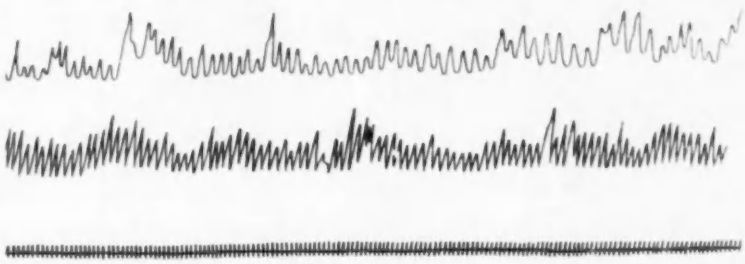


Fig. 3. Change in respiratory movements in Bolshoi on the 101st day after removal of the left cerebral cortex. Indications as in Fig. 1.

[2] R. Barsegyan, Fiziol. zhurn. SSSR 24, 6, 1043 (1938).

[3] A. V. Bondarcuk, Collected Scientific Works in Honor of A. L. Polenov [in Russian] (Leningrad, 1941) p. 107.

[4] N. N. Burdenko and B. N. Klosovskii, Vopr. neirokhir. 1,5 (1937); N. N. Burdenko, Collected Works (Moscow, 1950) 5, p. 201 [in Russian].

[5] S. D. Voznesenskii, Sovrem. psikhonevr. 7, 11, 293 (1928); Transactions of the First All-Union Congress of Neuropathology and Psychiatry [in Russian] (Moscow, 1929) p. 273.

[6] L. S. Gambaryan, Abstracts of Reports of the Conference on Problems of Evolutionary Physiology of the Nervous System [in Russian] (Leningrad, 1956) p. 49.

[7] S. N. Ivanova, Byull. éksptl. biol. i med. 45, 4, 39 (1958).•

[8] L. S. Isaakyan, Problems of the Physiology of the Central Nervous System [in Russian] (Moscow, 1959) 1, p. 229.

[9] B. N. Klosovskii, Vopr. neirokhir. 14, 4, 33 (1947).

[10] G. I. Markelov, The Autonomic Nervous System in Health and Disease [in Russian] (Odessa, 1935).

[11] M. D. Rutelburg, Voprosy 6, p. 42.

[12] B. D. Stefantsov, Fiziol. zhurn. SSSR 44, 8, 709 (1958).

[13] V. M. Ugryumov, Transactions of the All-Russian Scientific Conference on the Practice of Neurosurgery [in Russian] (Leningrad, 1954) p. 190.

[14] T. G. Urgandzhyan, Abstracts of Reports of the Conference on Problems of Evolutionary Physiology of the Nervous System [in Russian] (Leningrad, 1956) p. 162.

[15] T. Pill, The Neuro-Anat. Basis for Clinical Neurology (New York, 1954).

[16] G. F. Rossi and A. Brodal, J. Anat. 1956, V. 90, p. 42.

[17] D. Sheehan, Ann. Rev. Physiol. 1941, v. 3 p. 436.

•Original Russian pagination. See C. B. Translation.

CERTAIN BODILY REACTIONS TO COOLING THE BRAIN

N. V. Semenov, G. A. Chetverikova, and T. I. Konstantinova

Department of Normal Physiology (Head – Professor N. V. Semenov) Kalinin Medical Institute (Director – Docent A. M. Kushnev)

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 35-38, January, 1960

Original article submitted February 7, 1959

The widespread therapeutic application of hypothermia makes it necessary to undertake a broadly based study of the associated phenomena. The usefulness of the method is that by cooling a warm-blooded animal it is possible to interrupt the cerebral circulation for 10 to 30 minutes without causing irreversible changes.

In the last 10 years a large number of investigations of clinical and experimental hypothermia have been carried out. The principal changes taking place on cooling have been described in detail in both Russian and foreign publications [1-5, 7, 10-13, 15].

Experimental cooling is brought about by reducing the temperature of the surrounding air or by placing the animal in a cold water bath. Alternatively the blood may be cooled outside the body by passing it through a coil immersed in a cooling fluid (usually water and ice). The method of covering the animal with a special cooling blanket is also widely used.

Clinically, besides cooling, an anesthetic is also inhaled and many ganglion-blocking neuroplegic substances are given (the so-called "lytic cocktails"). These include promethazine, pethidine, scopolamine, succinyl choline, dimedrol, lidol (demerol), aminasine, promedol, and other substances. Their injection prevents harmful changes occurring and intensifies the action of the physical cooling. However, these substances are not without action on the organism. It has been shown that many complications occurring during cooling and during the recovering from hypothermia may be associated with the poisonous action of large amounts of the essentially toxic substances which make up the "lytic cocktail" [6].

It has been shown experimentally and clinically that for man and animals cooling is most successful at the so-called biological zero (about 30°). If the temperature falls below 26° harmful changes may occur in the cardiovascular and respiratory systems; these are frequently irreversible, and death ensues [3].

The shortcomings of the common method of attaining hypothermia make it necessary to search for new techniques. One approach which seems promising is the selective cooling of the cerebral blood supply, i.e., local cooling of the brain. In spite of its many advantages this method has not been widely used, and very few descriptions of it are available. It has been reported [4, 12, 14] that with this method there is comparatively little disturbance to blood circulation or respiration; it may be possible by these means to avoid the necessity for the injection of ganglion-blocking agents; the degree of anesthesia may be much less, and it may be possible to isolate the brain from the circulation for a sufficiently long period.

We have studied the relationship between the brain temperature and that of the rectum using different methods and degrees of cooling; we have also investigated the effect of the degree of hypothermia of the brain on the cardiovascular and respiratory systems, changes in the different sensory systems, as well as morphological changes in the brain tissue and particularly in its vascular supply.

METHOD

The experiments were carried out on dogs under morphine-ether anesthesia, and on rabbits under urethane. Blood clotting was prevented by injecting heparin. In dogs both carotid arteries, the vagal sympathetic trunks, and the femoral nerve and artery were exposed; in rabbits we used both carotid arteries, the sciatic nerve, and the tibialis muscle. A thermocouple was inserted to a depth of 8-10 mm into the brain through a trepanned hole. A second thermocouple was placed in the rectum. Canuli were inserted into the peripheral and central ends of the cut carotid artery and connected to a coil immersed in a vessel filled with ice. During the cooling, a clamp was placed on the common carotid of the opposite side. In dogs, the femoral artery was connected to a mercury manometer. In rabbits, movements of the tibialis muscle were recorded by means of a lever. The respiration was recorded by means of a hollow needle inserted into the trachea and connected to a Marie's capsule.

A thermocouple was placed at the output of the coil to control the extent of cooling of the blood pass-

ing rostrally in the common carotid artery. Forced circulation was used to speed the cooling.

RESULTS

Experiments on dogs. In all, 25 tests on dogs of both sexes and various weights were carried out: 7 experiments were made at 25°, and 8 at from 25° to 20°. In all cases, the temperature in the rectum fell less than in the brain, remaining higher by 4-6°. In 4 cases the differences amounted to from 7-12°. This phenomenon was encountered only with our particular method of cooling in which the fall in body temperature does not result directly from the cooled blood which is passing directly to the brain. This was demonstrated by measuring the temperature of the blood in the internal jugular vein. There is reason to suppose that the fall in temperature of the body results from reflex control of the rate of trophic processes of the whole body (Fig. 1), the control being exerted by the brain which reduces the metabolic rate. According to our own* and published results, at the onset of cooling the arterial pressure increases by 1-25%.

After a transitory increase, during the cooling the arterial pressure in the brain fell from 120-140 mm of mercury to 900-100 mm, and in some cases when cooled to 25-20° the pressure fell to 70 mm. During recovery from hypothermia, the arterial pressure returned to normal. Six animals were exceptional, and in them the arterial pressure remained at the low level of 60-70 mm for a considerable time. Of these, 4 died, and the remaining two made a slow recovery and remained in a poor condition for a long time.

As the brain temperature fell to 27-25°, there was a weakening of the pressor response to stimulation of the central end of the femoral nerve or the central end of the cut vagus nerve, and also a reduced response from the vascular reflexogenous zones. On cooling the brain below 25°, there was a disturbance of the cardiovascular reflexes which was shown by a reduction in arterial pressure on stimulating the central end of the femoral nerve, and an increased pressure on stimulating the central end of the vagus, while pressure applied to the carotid sinus caused the blood pressure to rise. The response of the heart and changes in arterial pressure occurring in response to stimulation of the peripheral end of the vagus remained unaltered at all temperatures down to the minimum (–20°) which we employed.

Some changes in the pulse pressure occurred during hypothermia and during recovery from it. Usually, with maximal cooling it was reduced by 25-30%; with very rapid cooling when the gradient was above 0.2, the reduction was 50%; during recovery from hypothermia, the pulse pressure returned to its original level.† When the animals were in a poor condition after the hypothermia both the pulse and the arterial pressures were low (pulse pressure 8-12 mm), and this was interpreted as an unfavorable sign.

Experiments on rabbits. In all, 21 tests on adult animals of both sexes weighing from 2-4 kg were made. A total of 13 experiments was made in which the brain was cooled to 25°, and 8 with cooling from 25-20°. The principal purpose of the experiments was to enable changes in skeletal muscle occurring during brain cooling to be recorded. We measured the temperature changes in the brain and rectum, the threshold of stimulation of the tibialis muscle, its chronaxy, and the pessimum frequency for suprathreshold stimulation. In most experiments, with brain temperatures of 25° and below there was a reduced excitability to direct stimulation, the muscle chronaxy was increased on average from 0.18 to 0.38 m/sec and there was a reduction in the critical pessimum frequency from 100-200 to 60-80 cps. During recovery from hypothermia, these quantities returned to their original values. The changes we observed therefore did not differ from those occurring when the whole body is cooled. It would appear that the small degree of change observed in these experiments is due to the comparatively small functional alteration in the lower parts of the central nervous system. This circumstance represents one of the advantages of isolated brain cooling.

Histological studies. The brains of animals which succumbed shortly after the experiment and also those of animals which survived cooling and were killed 3-4 days afterward, were examined histologically.

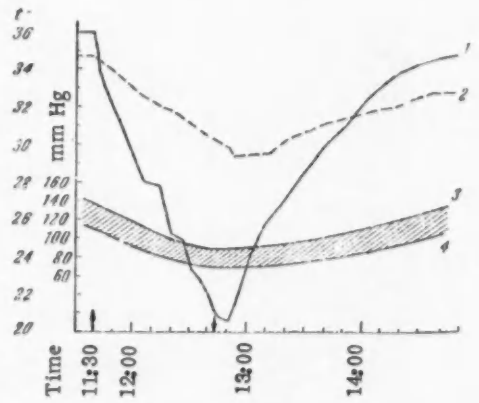


Fig. 1. Changes in temperature and arterial pressure in a typical experiment. 1) Brain temperature; 2) temperature in rectum; 3) maximal arterial pressure; 4) minimal arterial pressure. The onset of cooling is shown by an arrow (↑) and the end by the arrow (↓).

* N. N. Tkachenko assisted in some of the experiments.

† By the gradient of hypothermia we mean the number of degrees of temperature fall of the brain per minute.

The preparations were stained in Nissl's stain for nerve cells and by Eres' method for capillaries.

Investigations were made of the cerebral cortex, basal ganglia, and medulla. It was found that rapid cooling at a gradient greater than the 0.2 (which caused severe postcooling symptoms) was associated with many profound changes in the nerve cells. These were of various kinds; they were graded, and in some cases irreversible. In some cases the cells were swollen, there was often a perinuclear edema, the protoplasm was vacuolized, some of the giant pyramids were crenated and their nuclei eccentric. When the animal died shortly after the experiment, there was a pronounced perinuclear edema, the cells were strongly crenated, the apical outgrowths were greatly swollen, the Nissl substance was almost completely dissolved, and in some of the cells the protoplasm was vacuolized.

In spite of their diversity, all these changes can be included under the general heading "ischemic damage" [9]. We are led to this conclusion by the fact that in cases where there was well marked damage to the nerve cells there were also more or less well shown alterations to the capillaries, particularly in the medulla. On staining by Eres' method, it could be seen that the capillary plexus was much more reduced in those animals which died shortly after the experiment than in those which survived the hypothermia unharmed.

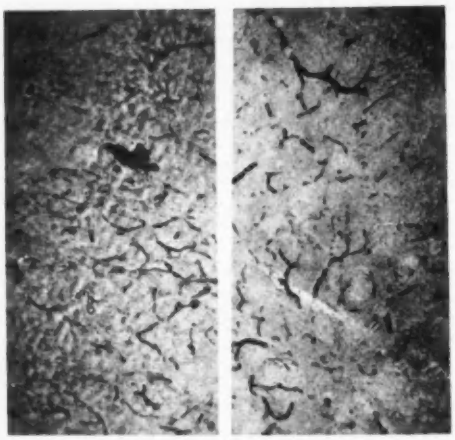


Fig. 2. Capillaries of the medulla in the dog. Stained by Eres, magnification 8 × 8. a) In an animal which tolerated cooling; b) in one which died shortly after cooling.

Figure 2 shows a comparison between drawings of the capillaries of an animal which tolerated hypothermia and another which died shortly after the cooling experiment. One of the causes of death from hypothermia is disturbance of compensatory regulation of function by the central nervous system. It appears that this functional disturbance is in large part brought about by disturbance of the cerebral circulation.

SUMMARY

Isolated cooling was applied to the brain of dogs and rabbits. When it was cooled to 20°, the body temperature did not drop below 30°. This favors the course of hypothermia. Respiration and cardiovascular tone is sufficiently maintained during the whole period of cooling. There is little change in properties of skeletal muscle. When low temperatures are reached, and particularly when the outcome is lethal, considerable changes are observed in the cerebral nerve cells and in the capillaries. The capillary changes may be the cause of those occurring in the nervous tissue.

LITERATURE CITED

[1] A. Labori and P. Yugenar, "Hibernation" Therapy in Medical Practice [in Russian] (Moscow, 1956).

[2] E. V. Maistrakh, The Theory of Anesthesia by Cooling [in Russian] Doctoral dissertation (Leningrad, 1955).

[3] G. A. Ryabov, Éksper. khir. 1, 25 (1958).

[4] B. A. Saakov, Hypothermia [in Russian] (Kiev, 1957).

[5] P. M. Starkov, The Problem of Hypothermia [in Russian] (Moscow, 1957) p. 5.

[6] E. Aron, Anesth. analg., 1954, v. 11, p. 399.

[7] D. R. Axelrod and D. E. Bass, Am. ges. Physiol., 1956, v. 186, p. 31.

[8] F. W. Behmann and E. Bontke, Arch. ges. Physiol., 1956, v. 263, S. 145.

[9] J. A. Corsellis, Brain, 1957, v. 80, p. 193.

[10] H. Hirsch and others. Arch. ges. Physiol., 1957, Bd. 265, S. 281, 314.

[11] H. Hirsch and others. Arch. ges. Physiol., S. 328.

[12] S. Kimoto, S. Sugie, and K. Asano, Surgery, 1956, v. 39, p. 592.

[13] J. Malmejac, P. Plane, and E. Bogaert, Compt. rend. Acad. sc., 1956, v. 242, p. 2764.

[14] W. M. Parkins, J. M. Jensen, and H. M. Vars, Ann. of Surg., 1954, v. 140, p. 284.

[15] H. L. Rosomoff, Am. J. Med. Sc., 1957, v. 234, p. 240.

THE ACTION OF SERA OF HEALTHY SUBJECTS AND ANEMIC PATIENTS ON ERYTHROPOIESIS

II. THE MECHANISM OF THE ERYTHROPOIETIC ACTION OF SERUM

S. Yu. Shekhter

Laboratory of the Physiology of Circulation and Respiration (Head – Professor G. P. Konradi), I. P. Pavlov Institute of Physiology of the AN SSSR and Clinic of Propedeutic Therapy (Head – Active Member AMN SSSR M. D. Tushinskii) I. P. Pavlov Leningrad Medical Institute

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 39-42, January, 1960

Original article submitted June 20, 1959

The erythropoietic influence of the serum of anemic animals and human subjects on rabbit blood has been demonstrated many times. It is sometimes thought that this effect is due to the appearance in the serum and plasma of so-called erythropoietins, but the evidence concerning the chemical nature and mode of action of the latter is very contradictory. Carnot and Deflandre [4], V. N. Shreder [2], Hodgson and Toha [10], and Rambach [13] think that their presence is associated with proteins. At the same time Borsook and his co-workers [3], Erslev and his co-workers [6, 7] Linman and Bethell [12], Gordon and his co-workers [9], and Fried and his associates [7] who obtained an erythropoietic effect after injecting a protein-free plasma extract, maintain that erythropoietins are not proteins. Korst and Bethell [11] and Gley and Delor [8] think that there are two erythropoietins one of which is a protien and the other not.

Finally, some workers [1, 2, 14, 15] deny the existence of special erythropoietins. Zih [14] ascribed the hemopoietic effect of the serum of anemic animals to the presence in it of dissolved hemoglobin. Ya. G. Uzhanskii [1] considers that stimulation of erythropoiesis in rabbits occurs secondarily through a hemolysis brought about by erythrodieretins which are present in large amounts in the serum of animals rendered anemic through bleeding.

At the suggestion of A. Ya. Yaroshevskii we have made a special study of the substances responsible for the erythropoietic effects of serum, and have tried to find whether the effect is primary or whether it results from the hemolytic action of the serum. The latter test was particularly important, since in our experimental work we never observed a single case where there was an increase in the amount of hemoglobin or of erythrocytes, in spite of the appearance of a reticulocytic crisis following the injection of many of the sera.

METHOD

The serum was freed from the globulin fraction by electrodialysis, the method being based on the property of globulin to precipitate in a salt-free medium. Distilled water was added to 3 ml of the serum to bring the volume to 10-12 ml, and the solution then poured into an ebonite chamber so that it lay in the space between two layers of parchment. A constant electrical current of maximum strength 150 ma from a 150-200 v source was continued until the current fell to zero through deposition of the electrolyte (after $1\frac{1}{2}$-$2\frac{1}{2}$ hours). The dialysate was filtered from the precipitated globulins, and after adding NaCl was injected into the rabbit at the same time that the serum of the same patient was injected into a second animal, as explained in a previous communication.

The presence in the serum and the titre of hemolysins was determined using the method of Thomson as modified by A. D. Zaslavskii. One drop of a 20% suspension of rabbit erythrocytes was added to 6 drops of whole or diluted plasma. The hemolysis was estimated visually after maintaining the mixture at 38° for 45 minutes.

Pigment metabolism was studied in rabbits using the usual method of Terven. Since no stercobilin was found in the feces, even after intravenous injection of distilled water which is known to be a hemolytic agent, we could determine only the amount of urobilin in the urine, although the presence of this pigment may result not only from an increased hemolysis, but also from liver dysfunction. The urobilin was estimated 1-2 days before and 3-7 days after injecting the serum.

RESULTS

In all, we carried out an electrodialysis of 20 sera from 15 anemic patients. Without giving detailed clinical descriptions of their condition, it may be mentioned that in 18 cases out of the 20 the effect of the injected dialysate was the same as that of the serum injected simultaneously; in 11 cases neither the serum nor the dialysate caused any reticulocytosis, in 7 both the serum and the dialysate caused an increase in the number of reticulocytes. Only in two cases had the serum treated by electrodialysis lost its reticulocytogenic properties. Both sera were taken from patients with secondary anemias (associated with cancer of the stomach and rheumatic endocarditis).

The fact that electrodialysis in most cases does not eliminate the reticulocytogenic properties of the serum indicates that the part played by the globulin in the hemopoietic action is secondary. The maintenance of the power of the serum to cause a reticulocytosis when the globulin fraction is removed indicates indirectly that the hemolytic action of the serum is not primary, because chemically the hemolysins are related to the globulins. Nevertheless we thought it necessary to make a special investigation of this point.

We therefore made a study in vitro to determine the presence of hemolysins acting on the erythrocytes of the patient or those of the rabbit. In 9 cases, not a single hemolysin active against the erythrocytes of the blood itself was found. The determination of hemolysins which acted on rabbit erythrocytes was carried out for 35 sera from 4 healthy human subjects and 25 patients. In all 35 cases, the serum completely dissolved rabbit erythrocytes, a result which accords with published results. At the same time, reticulocytosis occurred only with the injection of 13 out of 35 sera. We had to consider whether the reticulocytosis which occurs only in certain cases is due to the high content of hemolysins in the sera of the subjects. We therefore determined the titre of hemolysins in 19 sera from 16 patients. In 11 out of the 19 cases the value obtained was 1:4, and reticulocytosis occurred or was absent in the same number of cases irrespective of the titre.

Serum treated by electrodialysis lost the power of dissolving rabbit erythrocytes in vitro. Of the 10 dialysates studied by us of which 7 were taken from patients and 3 from healthy subjects, hemolysins were absent in all cases, whereas all the sera were reticulocytogenic.

Since the effect of the serum is independent of the titre of the hemolysins, and does not disappear when they are removed, we may explain the observed action of the serum only by assuming it is due to the introduction into the rabbit of hemolysins already elaborated.

We studied pigment metabolism * in 27 rabbits, into 17 of which injections from 4 healthy subjects and 12 anemic patients were injected. It was found that in all animals, before the serum injection, urobilin was either absent from the urine or present only in traces. Injection of healthy human serum never caused any increase in the amount of urobilin in the urine. At the same time, of the 17 injections of the serum of anemic patients, in 5 urobilinuria occurred.

However, this urobilinuria was never accompanied by a reticulocytosis, and in two instances the reticulocytosis actually occurred before urobilin was formed in the urine. The effect on urine urobilin of injecting dialysates was studied in 10 rabbits, and only in one animal was there an increase in the amount ofurobilin, although there was no reticulocytosis. At the same time we made obsevations on six rabbits in which the reticulocytosis caused by the injection of dialysate (2 cases) or serum (4 cases) was not accompanied by the formation of urobilin in the urine. The relationship between the amount of urinary urobilin and a reticulocytosis is shown in the table.

Relationship Between the Urobilinuria and Reticulocytosis

Number of rabbit	Amount of urobilin, mg	Effect of serum or dialysate.
56	63,3	+
69	21,6	—
58	32	Reticulocytosis preceded urobilinuria
68	79,8	—
94	20	Reticulocytosis preceded urobilinuria
67	294	—
75	—	+
79	—	+

The study of pigment metabolism has therefore shown that in some cases the serum of anemic patients has the property of causing urobilin to appear in the urine, but the fact that the urobilinuria occurs only occasionally and independently of the reticulocytosis shows that the reticulocytogenic effect of the serum cannot be ascribed to the increased hemolysis. It is possible that urobilinuria is a consequence of the damage to the liver occurring after the injection of the foreign serum.

The negative results obtained with the globulin fraction, with the introduction of complete hemolysins, and in studying hemolysis in the animal itself show that a primary hemopoietic effect is caused by injecting animals with serum from anemic patients. The absence of any parallelism between the increase in hemoglobin and erythrocytes when the number of reticulocytes increases may have been due to the small amounts of serum which we were compelled to use.

* A large amount of the work involved in observations on pigment metabolism was carried out by V. Ya. Plotkin, a student at the first I. P. Pavlov Leningrad Medical Institute.

SUMMARY

Experiments were performed on rabbits treated with serum from anemic patients. The nature of substances possessing erythropoietic properties was studied. It was found that the erythropoietic effect of the patients' blood was not related to the globulin fraction and also that it acted independently of any hemolytic agents present.

LITERATURE CITED

[1] Ya. G. Uzhanskii, The Part Played by Erythrocytes in the Regeneration of Blood [in Russian] (Leningrad, 1949).

[2] V. N. Shreder, Trudy inst. tsitologii, gistologii i embriologii 3, 1, 83 (1948).

[3] H. Borsook, A. Graybiel, G. Keighley, and others. Blood, 1954, v. 9, p. 734.

[4] P. Carnot and Cl. Deflandre, Compt. rend. Acad. sc., 1906, v. 143, p. 384.

[5] P. Carnot, Compt. rend. biol., 1906, v. 58, p. 344.

[6] A. J. Erslev and P. H. Lavietes, Blood, 1954, v. 9, p. 1055

[7] W. L. Fried, L. Pizak, L. O. Jacobson, and others, Proc. Soc. Exper. Biol. and Med. 1956, v. 92, p. 203.

[8] P. Gley and J. Delor, Compt. rend. Soc. biol. 1955, v. 149, p. 635.

[9] A. S. Gordon, S. J. Piliero, W. Kleinberg and others, Proc. Soc. Exper. Biol. and Med. 1954, v. 86, p. 255.

[10] G. Hodgson and J. Toha, Blood, 1954, v. 9, p. 299.

[11] D. R. Korst and F. H. Bethell, Clin. Res. Proc. 1957, v. 5, p. 142.

[12] J. W. Linman and F. H. Bethell, Blood, 1956, v. 2, p. 310.

[13] W. A. Rambach, H. L. Alt, and J. A. D. Cooper, Blood, 1957, v. 12, p. 1101.

[14] A. Zih, Arch. ges. Physiol., 1930, Bd. 225, S. 613.

FUNCTIONAL CHANGES IN THE CENTRAL NERVOUS SYSTEM IN TETANUS AND THE MODE OF ACTION OF THE TETANUS TOXIN

III. THE CENTRAL AND PERIPHERAL ACTION OF THE TETANUS TOXIN*

G. N. Kryzhanovskii

Laboratory of the Pathology of Infection (Head – Corr. Member AMN SSSR Professor A. Ya. Alymov) General Pathology Section (Head – Academician A. D. Speranskii) of the Institute of Normal and Pathological Physiology (Director – Active Member AMN SSSR V. N. Chernigovskii) AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 42-48, January , 1960

Original article submitted June 12, 1959

Despite the number of years for which it has been studied, much still remains unexplained concerning the mode of action of tetanus toxin.

There are three possible interpretations of its action. According to one [23, 24] the toxin acts on the motoneurones of the spinal cord which it reaches by passing along the motor nerves. Abel and his followers [14, 15, 25] deny that the toxin uses this path and consider that it enters the brain directly from the blood. A. V. Speranskii [12] and his co-wokers [2, 3, 4, 7, 8, 9] do not deny that the toxin enters the central nervous system but consider that its principal action is reflex.

One of the methods most widely used for determining the mechanism or action of the toxin is that of interrupting the sensory innervation of tissues into which the toxin is injected. This may be done either by injecting local anesthetic or by sectioning the corresponding dorsal roots in the spinal cord. The use of local anesthetic has given equivocal results [1, 7, 8, 13]. In addition, the results obtained are complicated by the extensive side effects of the drug on the nervous system.

Equally ambiguous results have been obtained when the sensory supply has been interrupted by a section of the dorsal roots [1, 13, 17-22, 26]. This is chiefly to be ascribed to the considerable methodological difficulties of carrying out this kind of experiment. On account of the structure of the spinal cord of the usual laboratory animals the operation of deafferentation inevitably causes considerable trauma and interference with the blood supply, so that when the operation is successfully performed various complications including locomotor disturbances or even complete paralysis of the hind limbs may occur. For this reason almost all the investigations referred to were carried out on a limited number of animals, and several authors [17, 18, 22] have used only one.

We decided to experiment on white rats which we had previously deafferented, and in which we had sectioned the motor roots to the hind limbs. In these animals, owing to the compact lumbar enlargement and the long dorsal roots of the spinal ganglia it is possible to carry out the operation without causing much trauma. The animals survived the operation quite well, and after section of the dorsal roots they usually showed no traumatic locomotor disturbances. This result is extremely important, because it enables us firstly to avoid any possible complications in the course of the disease which might produce misleading results in the experiment, and secondly to use a sufficiently large number of animals in the experiment.

METHOD

In all cases we deafferented the left hind limb. For this purpose, after removing the spinous processes of lumbar vertibrae I-III we removed the anterior articular process of vertibra III, the anterior and posterior articular processes of the II lumbar; and the posterior articular process of I lumbar; we also divided the pedicles between their origin and the base of the spinous processes (including the latter). In this way we exposed the left half of the lumbar enlargement and the central fissure of the spinal cord over only a limited area, and thus protected the cord from excessive trauma. We divided seven spinal roots: L_1–L_6, and S_1; in many cases, for purposes of control, we also divided the 13th thoracic and 2nd sacral roots. When the operation was correctly carried out, sensation was lost over the whole

* The principal results were presented at the Conference on Pathology of the AMN SSSR Institute of Normal and Pathological Physiology in April, 1955.

TABLE 1 The Action of Tetanus Toxin when Injected Intramuscularly into Normal Rats and Those with Deafferented Limbs

Number of group	Conditions of experiment	Number ofanimals in experiment	Number of animals affected with local tetanus
1	Toxin in gastrocnemius muscle of the deafferented left hind limb.	15	15
2	Toxin injected into gastrocnemius muscle of opposite (right) hind limb (left hind limb deafferented).	15	15
3	Toxin injected into gastrocnemius of mock-operated rats (spinal cord exposed).	15	15
4	Toxin injected into gastrocnemius muscle of left hind limb of unoperated rat (control for toxin)	15	15

limb from the digits to the thigh (as far as the inguinal fold).

The motor outflow was interrupted also on the same side by dividing the anterior roots of the same segments. When the operation had been correctly carried out, sensation was preserved over the whole limb, but there was a complete paralysis. Tests for sensation and mobility were applied twice, once a few days after the operation, and again before injecting the toxin. If there was any suspicion that some sensation remained after deafferentation, or movement after interrupting the motor outflow, the animals were not used for further experiments.

In the first set of experiments we studied the effects of deafferentation on the course of the illness (local tetanus) caused by injection of tetanus toxin. We used 1/20-1/25 of the M.L.D.(minimum lethal dose) contained in a volume of 0.05 ml. The toxin was injected into the gastronemius muscle 1-1½ weeks after the operation. In addition to this principal form of the experiment in which the toxin was injected into the deafferented muscle, we also carried out additional and control experiments to determine any possible effect of spinal cord trauma or of sensory loss on the course of the disease (Table 1).

In the second set of experiments we compared the effects of deafferentation and deafferentation on the course of tentanus induced by a lethal dose of toxin. The latter was given as two injections, one into the gastrocnemius muscle (1M.L.D. in 0.05 ml) and the other into the posterior group of the thigh muscles of the left leg (2 M.L.D. in 0.1 ml). To prevent the spread of toxin via the blood intravenously, we also injected antitetanus serum. The dose of the serum was 0.02 A. E. (antitoxic units) which was equal to about 2½ times that required to neutralize the total amount of toxin (3 M.L.D.) when injected directly into the blood. Because division of the motor nerves leads to muscular atrophy, it was shown in preliminary control experiments that the tetanus toxin injected into muscles of a deefferented limb does not become inactivated in the atropic muscles.

A special set of experiments was made to record the electrical activity in the left and right sciatic nerves. The record was made by a string oscillograph connected to the output of a two-channel amplifier. The potentials were led off from needle electrodes. These experiments were made at the end of the 1st and 2nd days after infection. In this last set of experiments 35 animals were used. The results were consistent, so that only the general result need be presented.

RESULTS

As is shown by the results presented in Table 1, injection of sublethal doses of tetanus toxin into the muscles of a deafferented limb caused a local tetanus in all cases without exception.

In the experimental group, the symptoms developed more slowly and were less severe than in the control animals. In all three control groups (Nos. 2, 3, and 4) the disease was at its height at the 3-4th day; there was no mobility in any of the joints of the limbs and all the muscles were rigid; in the experimental animals for the whole period of observation the mobility was preserved to some extent in the knee and hip joints. At the height of the disease, which was reached on the 4-6th day, there was a marked rigidity of the muscles of the lower leg, particularly of the gastrocnemius muscle into which the toxin was injected. Sometimes the muscles of the lower leg showed rigidity while those of the thigh were atonic, like a typically deafferented muscle.

These experiments showed therefore that division of the sensory portion of the reflex arc does not prevent the development of tetanus but only modifies its intensity owing to the changed tone in the deafferented muscles.

Injecting a lethal dose of tetanus toxin into the muscles of a deafferented limb in all cases caused an ascending tetanus to develop: This was at first local with a characteristic rigidity of the limb muscles; it then became segmental, and finally it became general and in most cases fatal (Table 2, Fig. 1).

There was no appreciable difference in the severity of the condition or in the number of animals dying (Group 1) and the corresponding controls (Group 3). We found merely that there was some lag in the development of the local tetanus in the experimental group on the 1st day.

The condition of animals receiving tetanus toxin into the muscles of the deafferented limb (Group 2) was very different. Not one animal in this group was affected by either local or general tetanus. Both before and after the injection of the toxin the deafferented limb remained in a paralyzed condition. No symptoms developed in the animals of the 2nd control group (No. 4) who received toxin injections into the blood together with the antiserum.

These results agree with those of other authors [20] who carried out a similar set of experiments.

Thus the interruption of the sensory innervation of a limb into which the toxin was injected does not prevent the development of either local or general tetanus. The development of the latter cannot be ascribed to the spread of toxin through the blood, because this was excluded by the failure of the disease to develop not only in control group No. 4 but in the experimental group with cut motor roots (Group 2). Complete protection from general tetanus was afforded only by section of the motor roots.

We must emphasize the total agreement between all experiments. There was no individual variation from one animal to another. This shows that the data obtained is completely reliable and that they demonstrate the conditions under which the disease can develop.

The result of the tests of the electrical activity of the sciatic nerves in the experimental and control groups (Fig. 2) agrees entirely with the results of the experiments described above. In the control rats which were not subjected to any operative measure, and also in those with a deafferented "tetanized" limb, during the 1st and 2nd days there was an increase in the electrical activity of the nerve in the injected limb. In rats with a deafferented left hind limb, neither left nor right sciatic nerve showed any increased electrical activity on either the 1st or 2nd day. In the left sciatic nerve the normal background potentials were absent.

The results of these experiments confirm those which we obtained previously [5, 6] and those of other authors [1, 2, 3, 16, 19, 27]; they show that the increased electrical activity in nerves during tetanus is of central origin and associated with the excitation of the corresponding motor neurones.

The results of all the experiments indicate therefore that the elimination of the sensory section of the reflex arc does not prevent the development of tetanus. It follows that the disease is not brought about by re-

TABLE 2 Animals Sick or Killed by Injecting Tetanus Toxin into the Muscles of Deafferented and Deefferented Limbs while Giving a Simultaneous Injection of Antitetanus Serum Intravenously

No. of group	Conditions of experiment	Number of of animals in experiment	Number of animals affected by tetanus	Number of animals dead from tetanus	Average length of life of animals which succumbed (days)
1	Toxin in muscles of deafferented limb, serum into vein	20	20	15	6.1
2	Toxin in muscles of deafferented limb, serum into vein	20	0	0	–
3	Toxin in muscles of normal limb, serum into vein	20	20	18	6.5
4	Toxin into vein, serum into vein	20	0	0	–
5	Toxin in muscles of a normal limb	20	20	20	2.3

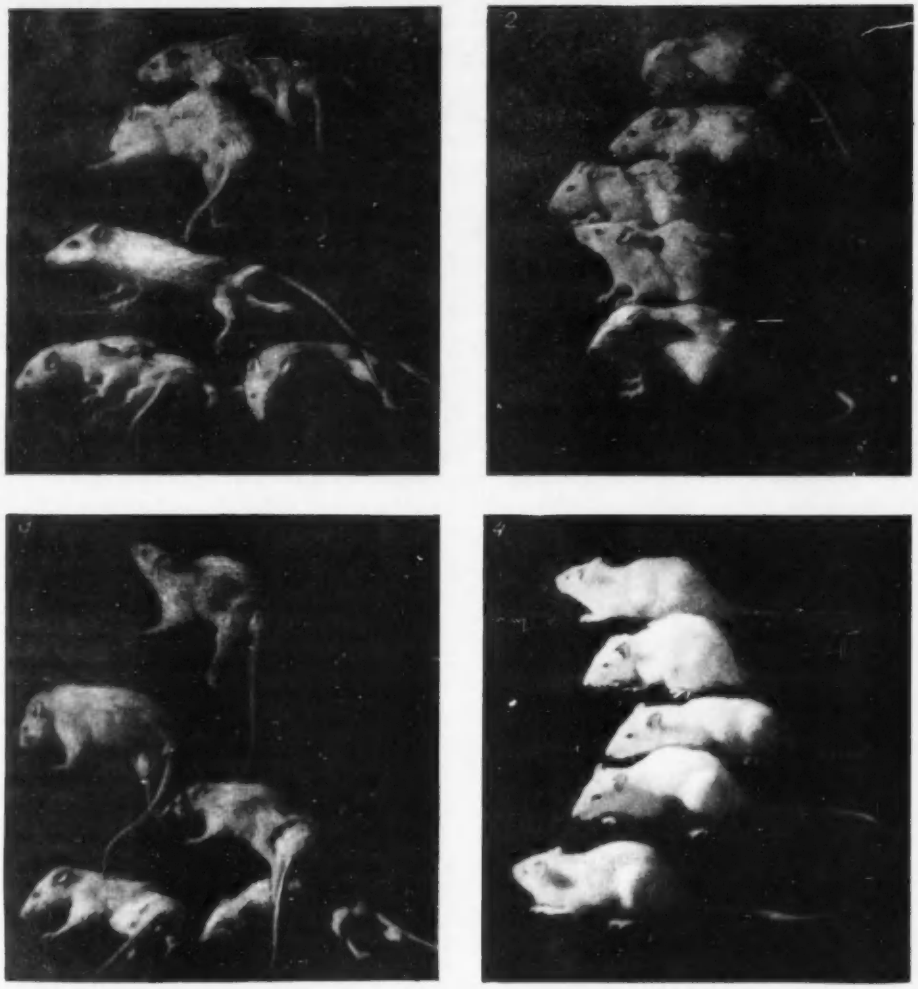


Fig. 1. Condition of animals on 4th day after injecting 3 MLD of tetanus toxin into muscles of a deafferented (1), a deafferented (2), and a normal (3) left hind limb, and after injecting toxin into the blood (4) while giving a simultaneous injection of antitetanus serum intravenously.

flex action. Our results confirm that an essential part of the action of the tetanus toxin is on the central nervous system. The fact that general tetanus is prevented by section of the anterior roots allows us to join with other authors [20] in saying that the main path of access of the toxin to the spinal cord is through the motor fibers.

Recently, in this laboratory, we and our associates have been able to obtain a direct proof of the fact that under normal conditions tetanus toxin enters the spinal cord principally if not exclusively by the ventral roots. A description of these experiments will be published separately and they agree entirely with those of the present work and confirm all the conclusions.

The role of the peripheral mechanisms in tetanus toxin may be to determine certain of the clinical features of the disease including changes in the reactivity of central nervous structures. This problem, however, requires to be investigated separately, especially because the published results are very much at variance.

It is important to emphasize that the fact that the toxin is able to move centrally along the nerve trunks is not denied by the supporters of the theory of its reflex action. A. D. Speranskii and his co-workers [10, 11, 12] not only pointed out that the tetanus toxin could enter the spinal cord, but also described many details of how this process was brought about.

Finally we must note that Abel's view [14, 15, 25] that general tetanus may develop only through the penetration of the toxin into the central nervous system from the blood contradicts much of the work which has been published and has not been confirmed by our experiments. The humoral pathway of the toxin may be of importance in determining the course and outcome of

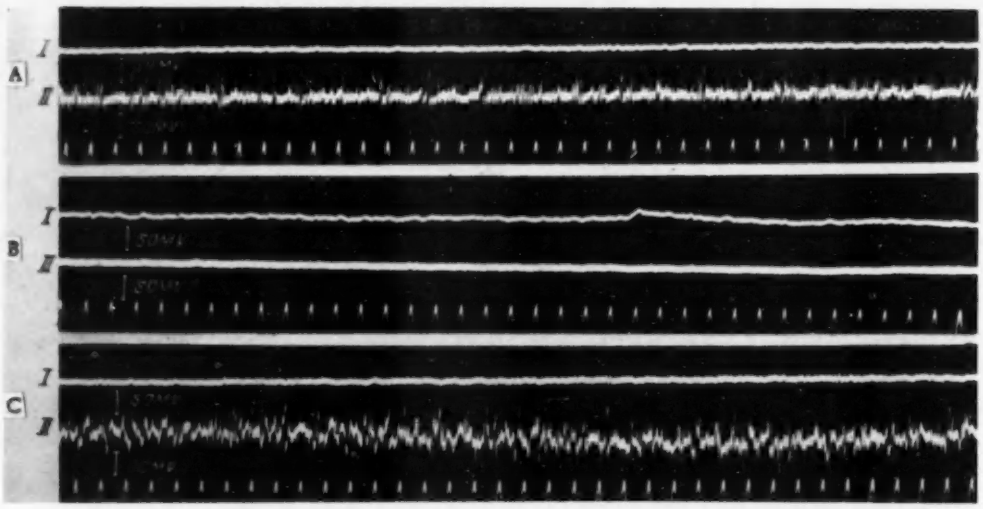


Fig. 2. Electrical activity in the right (I) and left (II) sciatic nerves one day after injecting toxin into the deafferented (A), deefferented (B), and normal (C) left hind limb while simultaneously injecting antitetanus serum into the bloodstream. Time marker (20 msec).

the disease, but is evidently not a necessary condition for its development. It is possible that the importance of this factor in the pathogenesis of the disease is different in different animals.

SUMMARY

Experiments were performed on white rats in which the sensory and motor roots were divided subdurally.

Deafferentation of one limb failed to prevent the development of a local tetanus following the injection of small doses of toxin into the muscles.

Lethal toxin doses injected into muscles of a deafferented limb while the humoral route of the toxin is blocked by antitetanus serum causes not only local but also general and fatal tetanus.

With the humoral route again blocked in the same way, lethal doses of toxin injected into the muscles of a deafferented limb do not cause a general tetanus.

In a deafferented injected limb, action potentials could be recorded from the sciatic nerve, but not from that of the deafferented limb. It is concluded that central mechanisms play a very important part in both local and general tetanus.

LITERATURE CITED

[1] N. V. Golikov, Research Reports of Leningrad University, Biological Series [in Russian] (1949) No. 16, p. 6.

[2] E. A. Gromova, Byull ėksptl. biol. i med. 36, 6, 29 (1953).

[3] E. A. Gromova, The Problem of Reactivity and Pathology [in Russian] (Moscow, 1954) p. 61.

[4] N. S. Delitsina, Trudy AMN SSSR (1952) 19, p. 254.

[5] G. N. Kryzhanovskii Experimental Physiological Analysis of the Mechanisms of the Development of Tetanus [in Russian] Candidate's dissertation (Moscow, 1952).

[6] G. N. Kryzhanovskii The Problem of Reactivity and Pathology [in Russian] (Moscow, 1954) p. 52.

[7] S. I. Lebedinskaya, Arkh. biol. nauk 41, 3, 114 (1936).

[8] O. Ya. Ostryi and G. N. Kryzhanovskii, Problems of Reactivity in Pathology [in Russian] (Moscow, 1954) p. 46.

[9] D. F. Pletsityn, Problems of Reactivity in Pathology [in Russian] (Moscow, 1954) p. 34.

[10] A. V. Ponomarev, Arkh. biol. nauk 26, 4-5, 211 and 220 (1926).

[11] A. V. Ponomarev, Arkh. biol. nauk 28, 1, 41 (1928).

[12] A. D. Speranskii, The Elements of the Structure of the Theory of the Medicine [in Russian] (Moscow, 1937).

[13] G. V. Shumakova, Problems of the Pathology of Infection, and Immunology [in Russian] (Moscow, 1954).

[14] J. J. Abel, Science, 1934, v. 79, p. 121.

[15] J. J. Abel and others, Bull. Johns Hopkins Hosp. 1935, v. 56, p. 84, p. 317; v. 57, p. 343; 1936, v. 59, p. 307; 1938, v. 62, p. 522, p. 610; v. 63, p. 373.

[16] G. H. Acheson, O. D. Ratnoff, and E. B. Schoenbach, J. Exper. Med., 1942, v. 75, p. 465.

[17] C. Brunner, Dtsch. med. Wschr., 1894, Bd. 20, S. 100.

[18] J. Cournont and M. Doyon, Arch. physiol. norm. et path., 1893, v. 25, p. 64.

[19] R. S. Davies, E. A. Wright and others, Arch. internat. Physiol., 1954, v. 62, p. 248.

[20] U. Friedemann, A. Hollander, and I. M. Tarlov, J. Immunol., 1941, v. 40, p. 325.

[21] A. Goldscheider, Ztschr. klin. Med., 1894, Bd. 26, S. 175.

[22] A. Gumprecht, Dtsch. med. Wschr. 1894, Bd. 20, S. 546.

[23] A. Marie and V. Morax, Ann. Inst. Pasteur, 1902, v. 16, p. 818.

[24] H. Meyer and F. Ransom, Arch. exper. Path. u. Pharmacol., 1903, Bd. 49, S. 369.

[25] W. Penitschka, Arch. klin. chir., 1953, Bd. 274, H. 5, S. 435.

[26] S. W. Ransom, Arch. Neurol. a Psychiat., 1928, v. 20, p. 663.

[27] E. A. Wright, R. S. Morgan, G. P. Wright and others, Lancet, 1952, v. 2, p. 316.

THE BARRIER FUNCTION OF THE LYMPH NODES IN INFECTION

N. V. Medunitsyn

Department of Pathological Physiology (Head – Corr. Member AMN SSSR Professor A. D. Ado) M. I. Pirogov Moscow State Medical Institute

(Presented by Active Member AMN SSSR N. N. Sirotinin)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 49-53, January, 1960

Original article submited March 30, 1959

It has been reported [8, 9] that the lymph nodes are able to fixate up to 79-99% of bacteria entering via the lymph vessels. Barrier function increases considerably during the process of immunization [1, 2].

It is well known that fixation of substances occurs in an inflamed area. The fixation results from coagulation of the plasma at the center of the inflammation, and also through thrombosis [4] and spasm of the lymphatic vessels [6].

D. A. Zhdanov [3] paid particular attention to the barrier function of the lymph nodes. In inflammation of the regional lymphatic nodes, reactive changes always occur. There is a swelling of the cells of the endothelium of the sinuses, infiltration of the node by granulocytes, hyperplasia of the lymphoid elements, which sometimes completely cover the sinuses of the node [7]. It is quite possible that similar nonspecific phenomena may be involved in increasing the barrier function of the lymph nodes in immunization.

In the present investigation we have studied the fixation of B. coli commune of the popliteal lymph node in healthy immunized animals and in others in which inflammation of the knee had been induced.

METHOD

The experiments were made on 36 cats and 6 dogs. The lymphatic nodes were perfused by a suspension of B. coli. A study was made of the lymph taken at operation from the lymphatic nodes in cats, and from a fistula of the thoracic trunk and lymphatic vessels of the hindlimb in dogs.

The animals were anesthetized by a subcutaneous injection of 50 mg per kg nembutal.

The perfusion fluid was prepared as follows: A 24-hour culture of B. coli (washed from an agar slant) was diluted in sterile Tyrode's solution to make 10^9 bacteria per milliliter, and then diluted to a strength of $5 \cdot 10^6$/ml. An accurate determination of the number of bacteria (both in the original and in the perfusion fluid) was made on plate cultures.

The lymph nodes were perfused for 20 minutes at a constant pressure of 20 cm of water. The barrier function was measured in terms of the percentage bacteria fixated.

Cats were immunized by giving three injections of heated B. coli at intervals of 5 days. One group was given an injection of 500 million bacteria into both hind feet, while the other group received 1 billion bacteria subcutaneously on one side of the body surface. The perfusion of the popliteal nodes was made on the 9-12th day after immunizing.

RESULTS

As is shown in the figure and in Table 1, the percentage B. coli fixated by the nodes in healthy animals when perfused varies over a considerable range (13.3-96.1). With immunized animals, and particularly in the lower leg, the variation in the percentage taken up varies less, while the average number is greater.

To determine the part played by the nonspecific component in the barrier function of the lymph nodes we induced an inflammation in the foot, and 2-5 days

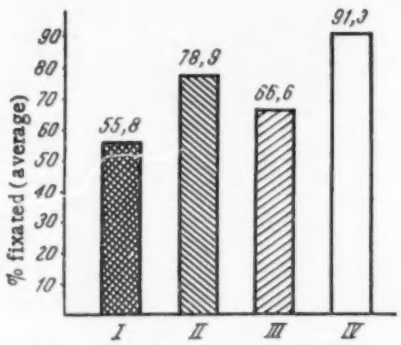


Fig. 1. Fixation of B. coli commune by the popliteal lymph nodes in cats. I) Healthy animal; II) animals immunized in the lower leg; III) animals immunized by injection into body side; IV) animals with inflammation in lower leg.

TABLE 1 Fixation of B. coli Commune by Popliteal Lymphatic Nodes in Cats

Group of animals	No. of animals	Number of perfused nodes	Limits of variation of percentage fixated	Standard error*	
				1	2
Intact animals	17	24	13,3—96,1	—	—
Immunized by injection into lower leg	7	12	45,2—97,1	$p<0,01$	—
Immunized by injection into body side	6	8	30,4—85,0	$p<0,01$	$p<0,1$
With inflammation of limbs	19	28	72,1—99,9	$p<0,01$	$p<0,01$

*This refers to the difference of the percentage fixated in the different groups: 1) with respect to percentage fixated in intact group, 2) with respect to percentage fixated in group immunized by injection in lower leg; $p < 0.01$ — statistically significant, $p < 0.1$ — not statistically significant.

later perfused the nodes with a suspension of B. coli. The inflammation was induced by injecting into the footpads either 0.1 ml of turpentine, or a suspension of 250 million staphylococci, or a centrifuged 2-day meat-peptone broth culture.

It was found that the variation in the percentage bacteria fixated was quite small, and that the average percentage was higher than in the healthy and in the immunized cats. The highest percentage taken up occurred when the inflammation was caused by the staphylococcal culture, it was less with the meat-peptone broth, and still lower when turpentine was used.

The experimental arrangement was now changed in order to eliminate certain defects in the method of perfusion. A cat in which inflammation had been induced in one hindfoot received an injection of 1 billion bacteria in 0.5 ml into both feet. In view of the report [6] of the possible functional spasm of the lymphatic vessels lying proximal to the inflammatory edema, the culture was injected 4-5 cm above the inflamed area. Up to 2-3 hours, lymph was collected into a sterile vessel from the afferent and efferent vessels of the popliteal lymph node, and then cultured.

It can be seen from Table 2 that a greater number of bacteria are fixated by the lymph node on the inflamed side.

We also carried out experiments on dogs. In 3, we caused an inflammation in the lower leg on both sides by injecting 1 ml of a centrifuged meat-peptone staphylococcal culture. After 3 days, polyethylene canulae were introduced into the thoracic duct and into the lymphatic vessel of one of the hindlimbs, and after one day 5 milliard B. coli in 2-2.5 ml of fluid were injected into both hind feet. The dogs with inflammation of the limbs received the injection 4-5 cm above the inflamed area, and the control animals were given the injection in the corresponding place. Lymph was collected with aseptic precautions 2, 6, 12, 24, and 48 days later.

Table 3 shows the results of experiments on 6 dogs (3 healthy and 3 with inflammation of the lower leg). In the first group there were more bacteria in the

TABLE 2 Count of B. coli Commune in Afferent and Efferent Vessels of Cat Popliteal Lymph Node

Number of animal	In inflamed limb		In healthy limb	
	Number of bacteria per ml of lymph			
	flowing in	flowing out	flowing in	flowing out
1	2 015 000	24 709	780 000	13 000
2	2 600 000	500	2 275 000	508 500
3	1 755 000	9 750	1 170 000	195 000
4	975 000	1 400	520 000	325 000
5	3 575 000	58 500	2 210 000	260 000
6	1 170 000	700	1 950 000	780 000
7	637 000	400	1 066 000	19 000

TABLE 3 Count of B. Coli Commune in Lymph of Healthy Dogs and Dogs with Inflamed Hind Limbs

Condition of animal	Number of animal	Number of bacteria per ml of lymph									
		After 2 hours		After 6 hours		After 12 hours		After 24 hours		After 48 hours	
		in vessel	in duct	in vessel	in duct	in vessel	in duct	in vessel	in duct	in vessel	in duct
Normal	1	140 000	1 950	650 000	5 460	780 000	7 800	416 000	55 250		
	2	221 000	6 760	1 170 000	13 000	988 000	13 520				
	3	299 000	2 080	1 950 000	8 450			325 000	5 330	10 400	130
Inflamed	4	198 000	0	845 000	0		0		0		0
	5	312 000	120	1 040 000	390			364 000	0	66 300	0
	6	91 000	0	624 000	0			71 500	0		

thoracic lymph duct; in the second group the lymph was sterile in most cases. In both groups, the number of bacteria in the lymph of the lymphatic vessels was approximately the same, there is therefore a smaller spread of bacteria in the second group, and this may be due to an increased fixation by lymph nodes modified as a result of the previous inflammation.

There are however indications [5] that in the first few hours after infection, owing to the increased lymph flow, the barrier function of the lymph nodes is not increased but on the contrary diminished. In our experiments where the lymph nodes were perfused at a late stage in the inflammation the fixation remained high even when the perfusion pressure was increased 2-3 times. Therefore increasing lymph flow at the onset of inflammation may cause a spread of the bacteria, but at later dates, reactive changes in the node may change it from a passive filter into an effective barrier against invading bacteria.

When there is inflammation, an increased mechanical filtration by the node may be observed. The weight of the inflamed lymphatic nodes is $1^1/_2$-3 times greater than that in normal animals. Histological investigation reveals a marked hyperplasia of the cells of the node. Also, a study of the cells in the efferent lymphatic vessels of a node revealed a large number of granulocytes, and a marked phagocytosis when the staphylococcal culture was injected. These nonspecific phenomena may play an important part in increasing the barrier function of the nodes during the process of immunization. The results obtained agree with those of V. M. Berman and E. N. Slavskaya [2] who consider the barrier function of the whole body to be made up of two parts, a nonspecific phase, caused by the inflammatory reaction at the site of entry of the bacteria, and a general reaction to the parenteral injection of the antigen.

SUMMARY

Fixation of B. coli commune by the popliteal lymph nodes was studied as follows: Lymph nodes were perfused by a suspension of B. coli commune, and studies were made of lymph taken at operation by means of fistulae in the thoracic duct and lymphatic vessels of a dog's hind limb. The ability of the lymph node to fixate bacteria is increased by immunization, particularly when the immunizing culture is injected into the area corresponding to the given node. The barrier function of the nodes increases, particularly when inflammation is provoked in them either by infectious or noninfectious agents. The nonspecific phenomena, which include intensification of the mechanical filtration and increase of phagocytic activity of the cells of the node play a significant part in the increase of lymph node barrier function in immunization.

LITERATURE CITED

[1] M. M. Barats, The Effect of the Functional Condition of the Nervous System on the Barrier Function of the Lymphatic Nodes [in Russian] Authors' abstract of dissertation (Ufa, 1957).

[2] V. M. Berman and E. M. Slavskaya, Problems of Reactivity at Different Ages and Infectious and Immunological Processes [in Russian] (Leningrad, 1955) p. 56.

[3] D. A. Zhdanov, Vrach. delo, 7-8, 393 (1946).

[4] V. Menkin, Inflammatory Changes [in Russian] (Moscow, 1948).

[5] N. S. Okun', Vestn. khir 63, 5-6, 27 (1943).

[6] Rusn'yak, et al., The Physiology and Pathology of the Lymphatic Circulation (Budapest, 1957) p. 523.

[7] R. Bieling and others, Handbuch der allgemeinen. Pathologie. Berlin, 1956, Bd. 7, t. 1.

[8] C. K. Drinker, M. Field, and H. K. Ward, J. Exper. Med., 1934, v. 59, p. 393.

[9] J. G. Widdicombe, R. Hughes, and A. J. May, Brit. J. Exper. Path., 1955, v. 36, p. 473.

THE EFFECT OF SODIUM AMYTAL ON THE PASSAGE OF SUGAR FROM ARTERIAL BLOOD INTO TISSUES OF THE HIND LIMB AND BRAIN

C. G. Genes and P. M. Charnaya

Department of Pathophysiology (Head – Honored Scientist Professor C. G. Genes) Ukranian Institute of Experimental Endocrinology (Director – Senior Scientific Collaborator S. V. Maksimov)

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 54-58, January, 1960

Original article submitted November 24, 1958

It is known that certain doses of anesthetic produce histological anoxia and reduce oxygen utilization [2]. Ether [10], Sodium amytal, and other substances also possess this property [14].

A consequence of the reduced oxygen utilization by the tissues is that the breakdown of glucose is also reduced. Some authors [1] have attributed the hyperglycemia which occurs under ether anesthesia to reduced oxygen utilization, although this also occurs under sodium amytal without any increase in blood sugar level [12].

If anesthetics reduce carbohydrate and oxygen utilization by the tissues, then it would be expected that there would also be reduced passage of sugar from arterial blood into the tissues.

We have studied this problem in dogs and have found to what extent sodium amytal influences the amount of sugar passing from the blood into the tissues of the hind limb (as constituting the principal mass of tissue) and brain, where utilization of the blood glucose is of special importance [5, 6, 16, 17].

METHOD

The experiments were carried out on healthy dogs under deep sodium amytal anesthesia. Repeated injections of a 2.5% solution of sodium amytal were given: the first injection was given subcutaneously, the remainder intravenously as required.

As an example, a dog weighing 24 kg received sodium amytal injections as follows: at 8 a.m. – 50 mg/kg; at 8:30 a.m. – 6 mg/kg; at 8:40 a.m. – 4 mg/kg; at 9 a.m. – 3 mg/kg; at 10 a.m. – 3 mg/kg; at 11:10 a.m. – 6 mg/kg; at 12:30 p.m. – 3 mg/kg; at 2 p.m. – 3 mg/kg; at 3:20 p.m. – 3 mg/kg, and at 7:20 p.m. – 2 mg/kg. When the animals were deeply anesthetized, and the corneal reflex could no longer be obtained, an opening in the skull in the region of the superior longitudinal sinus was cut by means of a trepine; the femoral artery and vein were exposed, a laparotomy was performed and the caudal portion of the pancreas removed; these dogs served as controls for the remaining experiments. The blood sugar level was determined using the method of Hagedorn-Jensen: This was done three times every 5 minutes before, and once hourly for 12-13 hours after laparotomy. Blood for examination was taken simultaneously from the femoral artery and from the superior sagital sinus. The normal body temperature was maintained by wrapping the animal up in blankets and warming with an electric lamp.

RESULTS

The Effect of Sodium Amytal on the Passage of Sugar into the Tissues of the Hind Limbs (Table 1)

A prolonged deep anesthesia induced by injecting sodium amytal 7-10 times over a period of 13 hours caused no change in the blood sugar level. The level fell only from 93-108 mg% at the beginning of the experiment to 80-85 mg% at the end, and this change could be ascribed to the 33 hours fast (the animals fasted for 20 hours before the experiment).

The passage of the sugar from the arterial blood into the tissues of the hind limb varied during the experiments over quite wide limits, and had an average value of 6 mg%. The transfer coefficient, which represents the ratio of the level in the tissue fluid to that in the blood, had a value of 7% in dog No. 8 and 6.6% in dog No. 9. In three determinations of the arteriovenous difference in blood sugar level in 6 dogs under deep sodium amytal anesthesia, the average tissue fluid concentration was 5.4 mg%, the blood sugar 86 mg%, giving a transfer coefficient of 6.3%.

These results differ little from those we obtained [5, 6] in healthy active dogs where the tissue sugar was 4.7 mg% and the transfer coefficient 5%.

TABLE 1 Blood Sugar Level and Amount (in mg%) Passing into Tissue in Animal Under Sodium Amytal Anesthesia

Before operation, min			After operation, hour													
0	5	10	1	2	3	4	5	6	7	8	9	10	11	12	12½	13	13½
Dog No. 8. Blood sugar level (artery)																	
93	92	91	92	91	89	84	79	84	88	90	91	78	88	79	82	80	
Passage of sugar into brain																	
11	17	15	22	15	22	22	15	13	9	20	20	26	24	16	18	14	
Passage of sugar into tissues of hind limbs																	
6	10	9	7	7	4	+1	5	6	8	16	+2	8	+1	11	3		
Dog No. 9. Blood sugar level (artery)																	
108	110	108	101	91	90	77	83	83	85	88	88	90	88	90	—	88	85
Passage of sugar into brain																	
19	16	18	11	12	11	11	16	15	17	20	20	18	14	14	—	22	17
Passage of sugar into tissues of hind limbs																	
0	6	2	5	5	9	1	17	4	5	6	10	2	2	13	—	11	9

It was quite otherwise with dogs under deep ether anesthesia [3-5, 7-9]. The tissues of the hind limbs in these animals removed 11 mg% sugar from arterial blood. We require to know the cause of this difference. Unlike sodium amytal, ether considerably increases the blood sugar level (from 126 to 141 mg%), and the higher its concentration the greater the amount of glucose extracted by the tissues. The considerable increase in the passage of sugar from the arterial blood into the tissues of the hind limbs does not however contradict the view that ether reduces the utilization of glucose by the tissues. In order to form a correct estimate of the amount of blood sugar transferred to the tissues, it is necessary to compare normal conscious animals with those under ether anesthesia when both have the same blood sugar level. We found that in the waking state [3-8], increasing the blood sugar to 129 and to 152 mg% by injection caused the transfer into the tissues of the hind limb to reach values of 14 and 21 mg%, i.e., values which are higher than under ether anesthesia. The greater transfer of sugar to the hind-limb tissues in waking animals as compared with those under ether anesthesia when both have the same blood sugar level is shown by the fact that in the first group the transfer coefficients were 10.9 and 13.8 and in the second 7.1 and 8.7.

Thus ether reduces the passage of sugar into the tissues of the hind limbs, but does not suppress it entirely. Sodium amytal, however, administered over a period of 13 hours changes neither the blood sugar level nor the absolute nor the relative amount passing into the hind limb tissues. We obtained these results in dogs in which sodium amytal exerted no toxic effect. During the whole of the experiment the respiration, pulse rate and rectal temperature remained normal. Different results were obtained in animals to which sodium amytal was toxic: The pulse rate fell from 106 to 80 per minute, respiration from 12 to 5 per minute, and the rectal temperature from 39 to 28° (Table 2).

In dogs No. 5 and 6, the passage of sugar into the tissues of the hind limbs was less than in Nos. 8 and 9, the values being 4.3 and 2.8 mg%. The transfer coefficients were 5.1% for No. 6, and 3.3% for No. 5. In view of the fact that in both dogs the blood sugar level was normal (86 and 82 mg%), this reduced transfer coefficient indicates a reduced ability of the tissues to utilize blood sugar.

In one dog, the passage of sugar into the limb tissues was measured hourly for 24 hours with the animal under deep amytal anesthesia. During this period the rectal temperature remained normal at 38-39°, the respiration remained within normal limits being 22 at

the beginning and 16 per minute at the end; the pulse rate was 120-144 per minute. The blood sugar level in this dog at the end of the experiment was the same as at the start, the average value of 23 determinations being 83 mg%. However the passage of sugar into the tissues varied considerably; it had an average value of 2.5 mg% and a transfer coefficient of 3%. In this animal, judging from the reduced passage of sugar into the brain over a 4-hour period (see Table 2), sodium amytal had a somewhat toxic effect, which was not reflected in the rectal temperature or respiration, though it did affect the utilization of sugar by the tissues.

It can be seen therefore that sodium amytal given in doses sufficient to cause anesthesia but which do not cause any toxic effects does not interfere with the maintenance of a normal blood sugar level or with the normal transfer of sugar into the tissues; however when given in toxic doses the blood sugar level is not changed, but there is less transfer into the tissues of the limbs which represent the main tissue mass of the body.

Sodium amytal and the transfer of sugar into the brain (Tables 1, 2). The brain utilizes glucose almost exclusively [16, 17] and more intensely than do other tissues. Thus, the transfer of sugar from the blood to the kidneys in a healthy dog represents 1.8 mg%, into the intestinal wall – 3 mg%, into the muscle of the heart – 3.9 mg%, into the spleen – 4 mg%, into the limb tissues – 4.7 mg%, and into the brain – 8 mg% [5, 6]. The higher the arterial blood sugar level, the greater the amount of sugar passing into the brain [11, 12]. The extraction and utilization of blood oxygen by the brain also increases parallel with the passage of sugar into it [15]. The requirement of the brain for sugar and oxygen is reduced in human subjects when in a drug-induced sleep [13]. It has been shown in in vitro experiments that barbiturates applied to brain tissue reduce oxygen utilization. When glucose is added to the brain tissue, there is also an increase in the uptake by it of atmospheric oxygen. In the case of the intact animal, different results are obtained. The transference of sugar into the brain from arterial blood during amytal anesthesia is considerably more rapid than in the waking state.

As the results of Table 1 show, over a 13-hour period the transfer of sugar to the brain in dog No. 8 varied from 9 to 26 mg%, but in dog No. 9 from 11 to 22 mg%. The average extraction of sugar by the brain in both dogs was nearly the same – 17 and 16 mg%, but the transfer coefficient (which is also coefficient of utilization) was 18% in dog No. 9 and 20% in dog No. 8. This rapid

TABLE 2 Blood Sugar Level and Its Passage (in mg %) into the Tissues, Sodium Amytal Anesthesia

Before operation, min			After operation, hour												
0	5	10	1	2	3	4	5	6	7	8	9	10	11	12	13	13½
Dog No. 5. Blood sugar level (artery)																
89	93	89	94	87	83	76	83	81	81	85	89	80	87	85	85	86
Passage of sugar into brain																
20	20	14	9	13	12	12	12	9	5	7	12	4	4	4	0	0
Passage of sugar into tissues of hind limbs																
+2	0	15	+4	11	7	+9	+2	5	1	4	6	4	6	2	3	0
Dog No. 6. Blood sugar level (artery)																
94	96	83	87	82	70	78	76	75	74	74	82	81	83	90	92	
Passage of sugar into brain																
20	18	11	15	17	0	11	11	14	0	4	+1	+2	0	0	0	
Passage of sugar into tissues of hind limbs																
5	15	10	11	2	+4	1	2	10	7	4	1	+2	9	0	+2	

transfer of sugar to the brain also occurred in 7 healthy dogs during deep amytal anesthesia. The extraction of sugar from the blood by the brain in these dogs had an average value as determined from 21 measurements of 17 mg%, and the utilization coefficient was 20%.

In one healthy animal the transfer of sugar into the brain was studied hourly for 24 hours in an animal under deep amytal anesthesia. In spite of the fact that in one dog for a 4-hour period toxic symptons occurred which led to a marked fall in the transfer of sugar to the brain (4, 5, 1, 5 mg%) the average value was nevertheless 12 mg%, and the utilization coefficient 14.5%.

Thus with amytal anesthesia, sugar utilization by the brain remains at a high level for as long as 24 hours. It must be noted that even in the last hours of the experiment the brain continues to extract the same amount of sugar from the blood as at the start of the experiment. This was found not only during the 13-hour but also during the 24-hour experiment. Therefore deep amytal anesthesia does not affect the power of the brain to utilize blood sugar rapidly. These results were obtained in dogs on which sodium amytal had no toxic effect. If in any animal there was a fall in respiration rate or in body temperature, then the extraction of sugar by the brain was also reduced (see Table 2). From the results given in Table 2 it can be seen that in dogs Nos. 5 and 6 the transfer of sugar to the brain is greatly reduced, and may even cease entirely in the last 7 hours of the experiment. This affects the average value of the sugar transferred to the brain during a 13-hour period: 0.2 mg% in dog No. 5 and 7.4 mg% in dog No. 6 with coefficients of utilization of 11 and 9%, respectively.

Consequently, when amytal produces no toxic effects, the brain maintains its ability to extract sugar rapidly from the blood. However if amytal exerts a toxic influence on the body, then this function of the brain is markedly reduced, and may fail entirely.

SUMMARY

The transfer of sugar from the blood into the limb tissues was shown to increase when either of two anesthetics was administered; this was due to a raised blood sugar level. When the blood sugar level rises, its transfer into the limb tissues is more marked in healthy nonanesthetized animals than in those under ether; it is also greater in animals under ether anesthesia from whom the pancreas has been removed than in similarly operated nonanesthetized dogs. Prolonged amytal anesthesia has no effect on the blood sugar level or on its normal transfer into the limb tissues. In the case of toxic symptoms which include a reduced rate of respiration and a lowered rectal temperature and which result from amytal anesthesia, the transfer of sugar into the limb tissues is reduced. Prolonged amytal anesthesia not only does not prevent the extraction of sugar from the blood by the brain but even increases it (in comparison with the amount of sugar transferred to the brain of nonanesthetized animals). In animals, sugar extraction by the brain is markedly decreased or completely arrested during the toxic action of sodium amytal.

LITERATURE CITED

[1] E. L. Berezov, Research Reports of Saratov University [in Russian] (1927) 6, 2, 3.

[2] E. I. Van Lir, Anoxia and Effects on the Organism [Russian translation] (Moscow, 1947).

[3] C. G. Genes, Byull. éksptl. biol. i med. 7, 1, 81 (1939).

[4] C. G. Genes, Byull éksptl. biol. i med. 7, 2-3, 204 (1939).

[5] C. G. Genes, The Pathogenesis of Sugar Diabetes [in Russian] (Khar'kov, 1940).

[6] C. G. Genes, The Pathogenesis and Therapy of Sugar Diabetes [in Russian] (Khar'kov-Kiev, 1944).

[7] C. G. Genes, E. L. Lipkind, V. B. Levina et al., Theses of the Third Ukranian Congress of Physiology, Biochemistry, and Pharmacology [in Russian] (Dnepropetrovsk, 1939). p. 21.

[8] C. G. Genes, E. L. Lipkind, V. B. Levina et al., Fiziol. zhurn. SSSR, No. 2, 236 (1941).

[9] C. G. Genes, E. L. Lipkind, A. M. Moskalenko, et al., Vrach. delo, No. 7, 551 (1937).

[10] N. V. Lazarev, Anesthetics [in Russian] (Leningrad, 1940).

[11] P. M. Rappoport-Charnaya, The Effect of Insulin on Various Aspects of Carbohydrate Metabolism in the Brain [in Russian] Authors' abstract of dissertation (Khar'kov, 1952).

[12] A. L. Chute and D. H. Smythe, Quart. J. Exper. Physiol., 1939, v. 29, p. 379.

[13] W. Dameshek, A. Myerson, and J. Loman, Am. J. Physiol., 1934, v. 91, p. 113.

[14] L. S. Goodman and A. Gilman, The Pharmacological Basis of Therapeutics, (New York, 1955).

[15] H. E. Himwich and J. F. Fazekas, Endocrinology, 1937, v. 21, p. 800.

[16] H. E. Himwich and L. H. Nahum, Am. J. Physiol., 1932, v. 101, p. 446.

[17] W. G. Lennox, Arch. Neurol. a. Psychiat., 1931, v. 20, p. 719.

[18] J. H. Quastell and A. H. M. Wheatley, Biochem. J., 1932, v. 26, p. 275; 1933, v. 27, p. 1609.

THE INFLUENCE OF METHIONINE AND LIPOCAIC ON THE LIPID METABOLISM OF THE LIVER OF RATS IRRADIATED WITH X-RAYS

V. G. Vladimirov

S. M. Kirov Order of Lenin Military Medical Academy (Director – Prof. K. S. Kosyakov), Leningrad

(Presented by Active Member AMN SSSR S. E. Severin)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 59-62, January, 1960

Original article submitted May 25, 1959

In the animal affected by radiation sickness, disturbance of the lipid metabolism takes place, and one manifestation of this is the development of fatty infiltration of the liver [8, 10, 12]. This is partly due to deprivation of the liver tissue of glycogen. Fatty infiltration of the liver may also be due to toxic products formed during irradiation.

There are contradictory reports in the literature concerning the content of phospholipids in the liver in radiation sickness: In some investigations [4, 9, 15] no changes were found, whereas in others [11] a slight decrease was particularly obvious before death of the animal.

Particular attention must be paid to the role of the lipotropic substances methionine and choline, which are necessary for the synthesis of phospholipids and prevention of fatty infiltration of the liver, especially because the content of methionine and choline in the liver of irradiated rats is decreased [2, 16].

In the present research an attempt was made to normalize the lipid metabolism in the liver tissue of irradiated rats by administration of methionine and lipocaic, which have been used clinically with success in the treatment of liver disease. Interest in the action of lipocaic in this case is accounted for by the fact that, as shown by the work of S. M. Leites and his co-workers, lipocaic possesses an inhibitory action on the development of toxic fatty infiltration of the liver [5, 6]. The indices selected for study were content of total lipids and phospholipids in the liver of rats.

METHOD

Experiments were carried out on male white rats weighing from 150 to 230 g. The animals were given a single exposure to total irradiation with x-rays (dose 650 r, current 15 ma, voltage 180 kv, dose rates 9.16 and 12.13 r/min, filters 0.5 mm Cu and 1 mm Al).

To ensure better utilization of the methionine and increase in its lipotropic action, the methionine was administered in combination with aminopeptide (a solution of the products of enzymic hydrolysis of the proteins of ox blood, consisting of aminoacids and peptides of low molecular weight; nitrogen content – 300 mg%). The compounds were injected subcutaneously, immediately after irradiation and then every 48 hours, in doses of 90 mg methionine and 25 ml aminopeptide per kg body weight.

Lipocaic was given at the same time by mouth, as an aqueous solution, in a dose of 1.3 units/kg body weight.

The lipids were extracted by a mixture of chloroform and methanol [13]. These were estimated in condensed form by oxidation with a chromate mixture the excess of which was titrated iodometrically [1]. The lipoid phosphorus was treated by mineralization and then estimated by the Fiske-Subba Row method, and the phospholipid content was then calculated (as lecithin) by multiplying this figure by 25.

RESULTS

The lipid content of the liver of healthy rats varied in 11 experiments from 5.3 to 7.73%, the mean value being 6.44% ($\pm \sigma = 0.8$; $\pm m = 0.24$).

In irradiated animals (Table 1), accumulation of lipids in the liver in the course of development of radiation sickness could be detected after 3-6 days, and it was very pronounced on the 9th day (mean 9.2%) and statistically significant. On the 12th day of the disease the total lipid content in individual animals reached 10-11%. The liver of these rats was anemic, yellowish in color and very friable. Meanwhile, individual animals were seen in which the content of liver lipids did not exceed normal even on the 9th-12th day of the disease. The phospholipid content in the liver of healthy rats varied in 11 experiments from 3.07 to 4.75%, the

TABLE 1 Total Lipid Content (Per Cent) in the Liver or Irradiated Rats under Different Experimental Conditions

Experimental conditions	Elements of the variational series	Days after irradiation				
		1	3–4	6–7	8–9	11–12
No preparations given	N	10	10	10	9	11
	V	5.39–8.0	6.38–9.12	4.66–9.45	7.3–11.0	6.0–11.6
	M	6.5	7.1	7.5	9.2	8.87
	±σ	0.93	0.86	1.53	1.3	1.57
	±m	0.29	0.27	0.48	0.43	0.47
Methionine with aminopeptide	N	10	10	8	10	11
	V	5.6–9.5	7.1–8.6	5.1–8.7	5.3–9.3	6.2–11.6
	M	6.9	7.7	7.1	7.7	8.1
	±σ	1.2	0.43	1.05	1.4	1.65
	±m	0.38	0.13	0.36	0.44	0.5
Lipocaic	N	10	10	10	11	8
	V	5.3–8.1	3.88–7.3	4.36–7.35	4.22–9.0	1.8–10.9
	M	6.7	5.9	6.1	6.85	6.94
	±σ	0.85	1.2	1.0	1.88	1.5
	±m	0.26	0.38	0.32	0.56	0.5

mean value being 3,55% (± σ = 0,43, ± m = 0,13). In the initial period of development of radiation sickness (on the first to third day) it fell slightly (Table 2). At subsequent periods the fall in the phospholipid content became marked: On the 6th day the phospholipid content averaged 2,31%, and on the 9th day their content reached its lowest mean value (2,13%). In some animals, however, even those which died, no such fall was observed.

The administration of methionine with aminopeptide slightly reduced the deposition of total lipids in the liver on the 8th-12th day after irradiation, and by comparison with controls, the phospholipid content was increased in the irradiated animals.

The therapeutic effect of administration of methionine which is a methyl group donor in the formation of choline, very probably is to improve the conditions for the formation of new choline-containing phospholipids, and primarily of lecithin.. There are findings which show that good results are also obtained from giving proteins rich in methionine, and especially casein [17]. It was impossible, however, to completely normalize the lipid metabolism by administration of methionine and aminopeptide. This was evidently because of the lowering of the activity of the enzyme system responsible for the processes of transmethylation [3].

A better action was obtained from administration of lipocaic. In this case, on the 9th-12th day after irradiation, the mean figures of the lipid content in the liver of the rats (see Table 1) were considerably below those in the series of experiments without treatment. Lipocaic also had a favorable action, to a lesser degree than methionine with aminopeptide, on the normalization of the content of phospholipids in the liver (see Table 2). As has recently been shown [7], the mechanism of the lipotropic action of lipocaic consists of activation of the processes of oxidation of fatty acids, the conversion of lipids into glycogen and the increase in their removal from the liver. Provided that the intake of methionine or choline into the body is adequate, lipocaic also activates the phospholipid metabolism. This may, possibly, explain why lipocaic, when used in this case without other lipotropic agents, was more successful in preventing the development of fatty infiltration, whereas methionine was more effective in relation to phospholipid metabolism.

In a separate series of experiments on 75 animals, observations were made for a period of one month on the rate of survival of the irradiated rats.

Administration of methionine with aminopeptide had a beneficial effect on the rate of survival: This reached 40% by comparison with 8% in control experiments. It is possible that aminopeptide, being a protein preparation, played the role of a nutrient substance, sustaining the animals through the crisis of the radiation sickness, when their digestive powers were rather diminished.

No increase was observed in the rate of survival of the irradiated animals treated with lipocaic (12% survived). This, however, is not an argument in favor of its omission from combined treatment. Fatty infiltration of the liver seriously interferes with the normal function of this organ, and attempts to overcome it are completely justified.

TABLE 2 Phospholipid Content (Per Cent) in the Liver of Irradiated Rats

Experimental conditions	Elements of the variational series	Days after irradiation				
		1	3–4	6–7	8–9	11–12
No preparations given	N	10	10	10	10	10
	V	2,82—3,79	2,45—3,5	1,69—2,9	1,27—3,07	1,7—3,5
	M	3,21	2,87	2,31	2,13	2,54
	±σ	0,4	0,85	0,14	0,57	0,45
	±m	0,12	0,27	0,04	0,18	0,14
Methionine with aminopeptide	N	10	10	8	11	11
	V	1,9—3,6	2,57—3,5	2,42—3,55	2,3—3,6	1,55—3,69
	M	2,71	2,93	2,74	2,92	2,71
	±σ	0,5	1,65	0,3	0,43	0,57
	±m	0,16	0,52	0,1	0,13	0,17
Lipocaic	N	10	10	9	11	8
	V	2,92—3,55	2,7—3,85	2,25—3,08	1,92—3,2	2,15—3,37
	M	3,15	3,16	2,61	2,68	2,87
	±σ	0,23	0,89	0,09	0,4	0,4
	±m	0,07	0,28	0,03	0,12	0,14

SUMMARY

Following X-ray irradiation (650 r), the content of fat in the liver gradually rises, while that of phospholipids decreases. Administration of lipocaic greatly reduces the developmnet of fatty infiltration in the irradiated rats. Employment of methionine with aminopeptide has asimiliar, although less prounced effect.

The fall in the phospholipid content of the liver of irradiated rats, to which these substances were given, was less than that in the control irradiated animals.

Administration of methionine with aminopeptide also increased the percentage of surivival of the irradiated

LITERATURE CITED

[1] S. D. Balakhovskii and I. S. Balakhovskii, Methods of Chemical Analysis of the Blood [in Russian] (Moscow, 1953).

[2] M. P. Eleazarova, Vestnik rentgenol. i radiol., 2, 22 (1956).

[3] M. P. Eleazarova and V. S. Stepanova, Proceedings of Sections of the All-Union Conference on Medical Radiology, Section of Experimental Radiology [in Russian] (Moscow, 1956) p. 48.

[4] I. I. Ivanov, V. S. Balabukha, E. F. Romantsev, et al., Metabolism in Radiation Sickness [in Russian] (Moscow, 1956).

[5] S. M. Leites and T. S. Yakusheva, Arkh. patol. 4, 44 (1949).

[6] S. M. Leites, Uspekhi sovremennoi biol. 18, 2, 214 (1944).

[7] S. M. Leites, Problemy endokrinol. i gormonoterap. 1, 71 (1955).

[8] M. Ariell, Radiology, 1951, v. 57, p. 561.

[9] J. G. Coniglio, W. J. Darby, J. A. Efner and others, Am. J. Physiol., 1956, v. 184, p. 113.

[10] W. E. Comatzer, J. P. Davidson and O. D. Engelstad and others, Radiation Res., 1954, v. 1, p. 546.

[11] J. P. Davidson, C. Simonson, and W.E. Cornatzer, Fed. Proc., 1954, v. 13, p. 197.

[12] F. Ellinger, Radiology, 1945, v. 44, p. 241.

[13] J. Folch and others, J. Biol. Chem. 1951, v. 191, p. 833.

[14] E. Geiger, Science, 1950, v. 111, p. 594, Fed. Proc., 1951, v. 10, p. 670.

[15] E. J. King, Biochem. J., 1932, v. 26, p. 292.

[16] U. S. Kumta, S. U. Gurnani, and M. B. Sahasrabudhe, Current Sc., 1955, v. 24, p. 362.

[17] C. C. Lucas and J. H. Ridout, Canad. J. Biochem. a. Physiol., 1955, v. 33, p. 25.

THE SITE OF THE SYNTHESIS OF PROTEIN-BOUND IODINE IN THE THYROID GLAND

V. I. Levenson

Department of Pathological Anatomy (Head – Corresponding Member AMN SSSR Prof. A. I. Strukov) of the I. M. Sechenov First Moscow Medical Institute

(Presented by Active Member AMN SSSR A. L. Myasnikov)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 62-66, January, 1960

Original article submitted May 6, 1959

The process of synthesis of protein-bound iodine is a very important step in the formation of the hormone in the thyroid gland. This process embraces a whole series of successive enzymic reactions, in the course of which inorganic, ionic iodine, concentrated in the thyroid tissue, is oxidized to molecular iodine and takes part in substitution and oxidation reactions with the tyrosyl groups of the protein molecule, eventually forming hormonally active iodothyronines (throxin and tri-iodothyronine), entering into the composition of thyroglobulin [2, 7, 11]. The connection between this biochemical process and morphological structures, of great interest to the histophysiology of the thyroid gland, is still, however, uncertain.

METHOD

The present reserach comprises 3 series of experiments, carried out on male white mice weighing 150-200 g: 1) an autoradiographic investigation of sections of thyroid tissue containing colloid; 2) an autoradiographic investigation of sections not containing colloid; 3) a radiochromatographic investigation of protein hydrolyzates of the thyroid gland. In the first series of experiments (34 rats), the animals were injected intraperitoneally with 10 μC of NaI^{131} over a period of 2 minutes, 21 days before they were sacrificed. The thyroid gland was fixed in Carnoy's fluid and embedded in paraffin wax. Sections 5-7 μ in thickness were freed from wax and subjected to autoradiography by the method of covering with liquid emulsion (type R). The length of exposure was from 48 hours to 16 days at +4°. In the second series of experiments (17 rats) 10-20 μC of radioactive iodine was injected over a period of 2 minutes, 4 days before the animals were sacrificed. The thyroid gland was fixed in 10% formalin; sections were cut out to a thickness of 10-15 μ on a freezing microtome. Repeated treatment with 70° alcohol and water led to removal of the colloid from most of the follicles. After staining with hematoxylin-eosin, an autoradiographic investigation was undertaken (as in the first series) with prolonged exposure of the sections (up to 1 month). In the third series of experiments (8 rats) 20-25 μC of NaI^{131} was injected. Animals were sacrificed after 30 minutes to 1 hour (first group) and after 24 hours (second group).

The thyroid glands of each group (4 rats in each) were homogenized in 2-3 ml distilled water; the protein was precipitated by an equal volume of 10% trichloroacetic acid. The precipitate was centrifuged and washed, and suspended in 1 ml of borate buffer (pH = 8.4), (10-15 mg of crystalline trypsin, at a temperature of 38°, for 48 hours under a layer of toluene). The material was then extracted once only with 0.5-1.0 ml butanol at pH=1-2 acidfied with concentrated HCl). In volumes of 30-40 μl, the extract was treated by descending chromatography, in a system: butanol – CH_3COOH-H_2O (78:5:17, top layer) for 10-14 hours. The dried chromatograms were exposed for 10 days between two x-ray films and developed in the usual way. In order to identify the iodine- containing aminoacids, we employed the R_f ratios known from the literature [14] and we also used NaI^{131} and di-iodotyrosine as controls (the latter was developed with ninhydrin). The optic density of blackening along the length of the radiochromatograms was estimated by means of the MF-4 photometric apparatus.

RESULTS

In the first series of experiments an autoradiographic reaction was seen from 30 minutes to 1 hour after injection of radioactive iodine, in the form of rings around the follicles. After 24 hours continuous areas of blackening appeared, in the form of disks over the intrafollicular colloid. The reaction gradually became weaker and it became negative on the 21st day (Fig. 1).

The ring reaction is of great interest in the determination of the site of organic combination of iodine. Leblond and Gross [5] and also Pavlovic [9], Nandi et al. [8] interpreted it as the index of preferential distribution of protein-bound iodine in the epithelium of

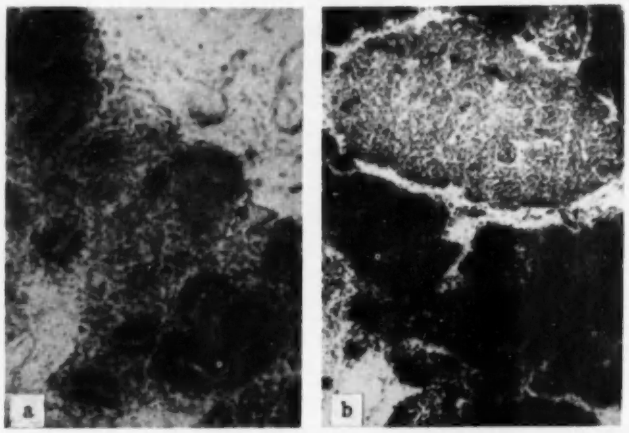


Fig. 1. Autoradiograms of paraffin wax sections of the thyroid glands of rats. Magnification: ocular 7× objection 10×. a) One hour after injection of I^{131}; b) after 24 hours.

Fig. 2. Autoradiogram of a frozen section of the thyroid gland of a rat (colloid removed) one hour after injection of I^{131}. Magnification: ocular 10 ×, objective 40 ×. An obvious but weak autoradiographic reaction is observed over the cells of the epithelium.

the follicles. However, Wollman and Wodinsky [15] and also M. F. Merkurov [1] have pointed out that in those follicles in which the colloid has become separated from the epithelium of the wall by the action of the fixing agent, the ring of the autoradiographic reaction does not correspond to the epithelial cells, but to the peripheral zone of the intrafollicular colloid. These authors consider that the iodization of the protein molecule is brought about in the colloid and not in the cytoplasm of the thyroid epithelium.

In our material the picture of displacement of the ring together with the peripheral zone of the colloid was repeatedly encountered. This gives grounds for considering that the iodized protein, producing this type of reaction, was localized in the colloid. It is, however, unjustifiable to conclude that the intrafollicular colloid plays an indispensable role in the process of organic combination of iodine, because of certain considerations which arise from the method itself. The autoradiographic resolution of such closely situated microobjects as the follicular epithelium and the follicular colloid is achieved by the use of fairly thin layers of emulsion and short periods of exposure. In these conditions, only areas of high activity or of higher concentration of radioactive isotope are found. If the concentration of organic I^{131} in the peripheral zone of the colloid is much higher than in the cells, then no autoradiographic reaction takes place over the cells. The autoradiographic investigation of paraffin wax sections thus gives no grounds for denying the presence of organic iodine in the epithelial cells of the thyroid gland.

In the second series of experiments, in which we made autoradiographic investigations of sections obtained by the freezing microtome, the colloid was removed from the sections by treatment with alcohol and distilled water. Completeness of removal of the colloid was controlled by histological examination. An obvious positive reaction over the cells was observed after prolonged exposure of the sections, considerably less in intensity than the corresponding autoradiograms of paraffin wax sections that were investigated (Fig. 2). These results show that the cells of the thyroid epithelium contain iodized protein, although in a considerably smaller concent3ation than the colloid.

There are three possible explanations of these facts: 1) the iodized protein enters the cells from the colloid, in which the process of iodization takes place; 2) the organic iodine is entirely synthesized in the cells and is reapily excreted into the colloid; 3) the process of iodization takes place in both cells and colloid.

The first hypothesis is contrary to the established mechanism of mobilization of the colloid, which is brought about by the proteolytic decomposition of the protein molecule and resorption of the decomposition products of low molecular weight [4, 10, 14, etc.]. Meanwhile, these products cannot form the substrate of the autoradiographic reaction, for they are washed from the section by the action of the histological reagents. The protein-bound iodine, thus detected autoradiographically in the cells, is an indication of the intracellular formation of iodothyroglobulin.

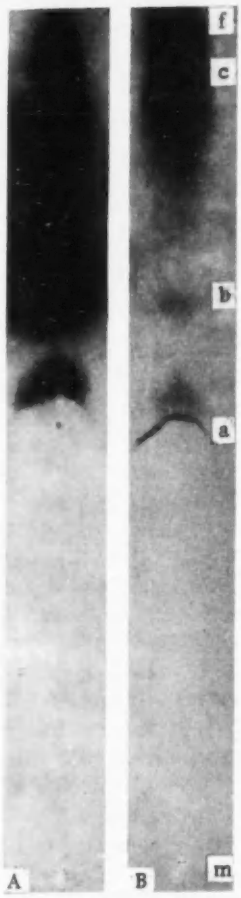


Fig. 3. Radiochromatograms of tryptic hydrolyzates of the thyroid glands of rats. In the period from 1 to 24 hours, the activity of the iodothyronines increases at the expense of a considerable fall in the activity of mono- and di-iodotyrosine.
A) One hour after injection of I^{131}; B) after 24 hours; a) Stain corresponding to MIT; b) stain corresponding to DIT (identified by control tests); c) stain corresponding to iodothyronine; M) line of application of hydrolyzate; F) front of solvent (34 cm).

Is the epithelial cell the only site for formation of the thyroid hormone, or does this process also take place in the intrafollicular colloid? The results of the third series of experiments, shown in Fig. 3, tend to support the latter. As was shown in the first series of experiments, at all times of the chromatographic investigation (from 1 to 24 hours) the organic iodine was present almost entirely in the colloid, although one hour after its administration it was mainly situated in the peripheral zone, and later on, almost uniformly throughout the whole colloid. Meanwhile, in the intervening period (from 1 to 24 hours) the proportion between the activity of the iodine-containing aminoacids of the thyroglobulin underwent an essential change: The activity of the iodothyronines (thyroxin and tri-iodithyronine - Tx and TIT) rose at the expense of a fall in the activity of mono- and di-iodityrosine (MIT and DIT). These findings agreed with the results of the extensive investigations of Roche and his co-workers, who found that the level of labeled MIT reaches its maximum 8-10 hours after injection of radioactive iodine, that of DIT a few hours later, that of TIT after 12-14 hours and that of Tx after 24 hours [12]. Thus, in the period between 8 and 24 hours when nearly all the labeled thyroglobulin is present in the colloid, condensation of the iodityrosines takes place, with the formation of iodothyronines and the conversion of MIT and TIT into DIT and Tx, i.e., the further iodization of the monoiodo-substituted tyrosine groups of the protein molecule. It is highly probable that combination of iodine with those tyrosine radicals of the protein molecule that were not, generally speaking, subjected to halogenization before secretion into the colloid, may take place also in the colloid.

SUMMARY

The author conducted an autoradiographic investigation of the thyroid gland at various intervals after the administration of radioiodine to rats. The method of liquid emulsion coating was used. Paraffin sections prepared at intervals of 30 minutes to 1 hour produce an autoradiographic ring reaction which, in the main, corresponds to the peripheral zone of the intrafollicular colloid. By using frozen sections devoid of the colloid the author established the presence of small quantities of protein-bound iodine in the thyroid epithelium. Radiochromatographic investigation of thyroid tissue hydrolyzates at different intervals after radioiodine administration points to the fact that, during the interval of 1-24 hours, when the bulk of the protein-bound iodine is in the colloid, the amount of radioiodothyronines increased at the expense of radioiodotyrosine. A conclusion is drawn that the formation of iodine-containing organic compounds in the thyroid gland begins in the epithelial cells and continues in the follicular lumen.

LITERATURE CITED

[1] M. F. Merkulov, Problemy éndokrinol. i. gormonoterap. 3, 6, 26-30 (1957).

[2] S. A. Berson, Am. J. Med., 1956, v. 20, p. 653.

[3] I. Doniach and S. R. Pelc, Proc. Roy. Soc. Med., 1949, v. 42, p. 957.

[4] A. J. Dziemian, J. Cell. a. Comp. Physiol., 1943, v. 21, p. 339.

[5] C. P. Leblond and J. Gross, Endocrinology, 1948, v. 34, p. 306.

[6] N. Miani-Padoue, Acta anat., 1956, v. 27, p.89,

[7] R. Michel, Ann. Rev. Physiol., 1956, v. 18, p. 457.

[8] S. Nandi, R. K. Poddar, and C. K. Pyne, J. Endocrinol., 1956, v. 13, p. 125.

[9] M. Pavlovi, J. Endocrinol, 1955, v. 12, p. 227.

[10] E. de Robertsi, Ann. New York Acad. Sc., 1949, v. 50, p. 317.

[11] R. W. Rawson, J. E. Rall, and M. B. Sonenberg, in the book: The Hormone. New York, 1955, v. 3, p. 433.

[12] J. Roche and R. Michel, Ann. Rev. Biochem., 1954, v. 23, p. 381-500.

[13] J. Roche, S. Lissitzky, and S. Michel, Biochem. et biophys. acta, 1951, v. 7, p. 257.

[14] P. Watson and V. Trikojus, Ann. Chemie, 1957, Bd. 607, p. 215.

[15] S. H. Wollman and I. Wodinsky, Endocrinology, 1955, v. 56, p. 9-20.

THE INFLUENCE OF SYMPATHETIC DENERVATION ON THE CONTENT OF GLYCOGEN AND HIGH-ENERGY PHOSPHORUS COMPOUNDS IN THE MYOCARDIUM

V. A. Govyrin

I. M. Sechenov Institute of Evolutionary Physiology (Director – Academician L. A. Orbeli*) of the Academy of Sciences of the USSR, Leningrad

(Presented by Active Member AMN SSSR S. V. Anichkov)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 67-69, January, 1960

Original article submitted June 12, 1959

According to Schumann, the energy resources of the heart, consisting mainly of glycogen, adenosinetriphosphoric acid, and creatinephosphate, are goverened by a complex neurohumoral mechanism (M. E. Raiskina [8], Raab [17]), and influences from the extracardiac sympathetic nerves play an important part in determining the functional condition of this mechanism.

Chang [12], E. S. Rozovskaya [10], Schumann [18], M. E. Raiskina [7], and Raab [17] showed that stimulation of the sympathetic nerves of the heart, as well as imitation of this effect by injection of adrenalin and noradrenalin, are accompanied by an increase in the work of the the heart and, at the same time, increased expenditure of energy, as a result of which there is a fall in the content of glycogen, ATP, and creatinephosphate in the myocardium.

According to Raab [17], stimulation of the sympathetic nerves and injection of adrenalin lead to a considerable increase in the catecholamines in the heart.

Slightly more complex, and, therefore, more difficult to explain, is the change in the content of high-energy compounds observed during stimulation of the augmenting nerve of Pavlov alone [2]. M. E. Raiskina [9] showed that stimulation of the augmenting nerve accelerates the renewal of the phosphorus compounds of the heart and that this effect evidently underlies its action on the heart.

Of great interest to the study of the mechanisms of the trophic influence of the sympathetic nervous system is the use of the method of sympathetic denervation in experiments of long duration. Information on this subject in the literature is scanty and often contradictory. The existing papers by B. B. Brandsbrug [4], F. M. Lampert [5], Jonnesco and Ionescu [14], N. A. Podkopaev [6], E. S. Shakhbazyan [11] and Long et al. [16], are devoted almost exclusively to the effect of sympathetic denervation, usually incomplete, on the structure of the heart and certain of its functional aspects.

Little work has been done on the biochemical changes in the heart muscle after sympathetic denervation. The work of Raab [17] and of Goodall and Kirshner [13] showed a sharp fall in the content of adrenalin and noradrenalin in the heart of cats and sheep after sympathetic denervation. Meanwhile, the elucidation of the relationship between the presence or absence of sympathetic influences on the heart and the content of energy-producing compounds therein may help towards the understanding of the mechanism of the tropic influences of the sympathetic nervous system on the heart. The present research is devoted to the study of this problem.

METHOD

For the experiments we used 10 cats in which the sympathetic nerve supply of the heart was completely removed (one-stage extirpation of both sympathetic trunks from the superior cervical ganglion to the 8th-9th segment of the thoracic chain), and 14 control animals. Investigations were carried out at various times from 20 to 110 days after sympathetic denervation. For the biochemical estimations, the apex of the heart was taken from cats, anesthetized with nembutal, and under artificial respiration, and the tissue rapidly frozen in liquid nitrogen. Glycogen was estimated by the method of Kemp and Kits van Hijningen [15], adenosine polyphosphates as readily hydrolyzed phosphorus, and creatinephosphate by M. A. Alekseeva's method [3]. In the course of the experiments, recordings of the electrocardiogram were made, and histological examinations were carried out on the hearts of operated and control animals.

RESULTS

Sympathetic denervation led to an obvious increase in the content of glycogen, adenosine polyphosphates and creatinephosphate in the heart. In spite of

*Deceased.

TABLE Content of Glycogen, Adenosine Polyphosphate, and Creatinephosphate in the Myocardium (in mg % of Crude Substance)

Serial No.	Time after operation (in days)	Glycogen	Adenosine polyphosphate as P	Creatinephosphate
	Normally			
1	—	548	Not determined	70,0
2	—	381	32,8	74,9
3	—	Not determined	19,6	85,2
4	—	456	26,6	78,5
5	—	Not determined	26,7	136,2
6	—	809	31,8	101,5
7	—	450	33,3	132,0
8	—	651	36,3	103,6
9	—	539	31,3	145,6
10	—	837	22,6	146,5
11	—	428	16,3	Not determined
12	—	653	27,2	151,9
13	—	716	24,8	194,4
14	—	—	27,2	176,8
	M±m	588±47,1	27,4±1,6	122,8±11,2
	After sympathetic denervation			
1	20	777	35,2	100,3
2	23	1 106	22,1	149,0
3	53	911	35,1	150,8
4	56	935	34,8	172,8
5	60	744	33,4	147,6
6	90	1 069	35,6	81,4
7	90	540	31,2	157,3
8	96	892	33,0	176,6
9	101	1 032	50,1	160,0
10	110	930	43,4	136,1
	M±m	893±55,0	35,4±2,3	143,2±9,1

individual variations in the content of these compounds, the average glycogen content of the myocardium for the group of operated animals was increased by 50%, that of adenosine polyphosphates by 30%, and that of creatinephosphate slightly. The results obtained were thus directly opposite to the effect of stimulation of the sympathetic nerves of the heart.

The raised level of glycogen and high-energy phosphorus compounds indicates changes in the energy metabolism of the heart, and evidently demonstrates a reduction in its intensity. It is still difficult to judge whether any connection exists between this phenomenon and the sharp fall in the content of adrenalin and noradrenalin in the heart after sympathetic denervation, which was observed by Goodall and Kirshner [13].

The increased content of glycogen and high-energy phosphorus compounds may still be detected in myocardium 3-3½ months after sympathetic denervation, i.e., at times when the level of adrenalin and noradrenalin, according to Goodall and Kirshner, had returned to their initial values once more.

It seems very unlikely to us that there is any connection between the increased content of these compounds and the slight decrease in the heart rate after sympathetic denervation.

This hypothesis is based on the experiments of M. E. Raiskina [9], who showed that the increase in the content of glycogen and high-energy phosphorus compounds was connected with a slowing in their renewal in the heart muscle after sympathetic denervation. We regard these phenomena as disturbances of the energy metabolism of the heart resulting from the abolition of the trophic influences of the sympathetic nerves of the heart.

SUMMARY

Complete desympathization of the heart provokes changes in the macroergasic metabolism of myocardium. In operated animals a rise is noted in the content of glycogen, adenosinepolyphosphates, and creatinephosphates. This phenomenon may be regarded as one of disturbances of the macroergasic metabolism of the

heart provoked by the exclusion of the trophic effects of the cardiac sympathetic nerves.

LITERATURE CITED

[1] I. P. Pavlov, Complete Collected Works [in Russian] (Moscow-Leningrad, 1951) Vol. 1, pp. 419, 577.

[2] N. M. Abramova, V. F. Anisimova, A. V. Gutovskaya, et al., Byull. eksptl. biol. i med. 44, 7, 50 (1957).

[3] A. M. Alekseeva, Biokhimiya 21, 2, 243 (1956).

[4] B. B. Brandsbrug, Transactions of the 17th Congress of Russian Surgeons [in Russian] (Leningrad, 1926) p. 105.

[5] F. M. Lampert, Transactions of the Twentieth Congress of Russian Surgeons [in Russian] (Moscow, 1929) p. 150.

[6] N. A. Podkopaev, Fiziol. zhur. SSSR 20, 2, 277 (1936).

[7] M. E. Raiskina, Farmakol. i. toksikol. 14, 1, 31 (1951).

[8] M. E. Raiskina, Uspekhi sovremennoi biol. 2, 173 (1952).

[9] M. E. Raiskina, Byull. éksptl. biol. i med. 41, 5, 44 (1956).

[10] E. S. Rozovskaya, Farmakol. i toksikol. 8, 1, 11-14 (1945).

[11] E. S. Rozovskaya, Experimental Data on Disturbance of the Coronary Circulation in the Heart [in Russian] (Moscow, 1940).

[12] I. Chang and J. Quart, Exper. Physiol., 1937, v. 26, p. 285.

[13] McC. Goodall and N. Kirshner, J. Clin. Invest. 1956, v. 35, p. 649.

[14] T. Jonnesco and D. Ionescu, Zeitschr. Ges. exper. Med., 1925-1926, Bd. 48, S. 516.

[15] A. Kemp and A. J. M. Kits van Hiijningen, Biochem. J., 1954, v. 56, p. 646.

[16] D. M. Long, R. C. Truex, K. R. Friedmann et al., Anat. Rec., 1958, v. 230, p. 73.

[17] W. B. Raab, in the book: Fortschritte der Kardiologie. (Basel-New York, 1956) Bd. 1, S. 65.

[18] H. Schumann, Der Muskelstoffwechsel des Herzens, Seine Physiologie, Pathologie and Klinik, Darmstadt, 1950.

THE FUNCTIONAL INTERACTION OF VITAMINS C, P, AND B_1

E. F. Shamrai and T. V. Fetisova

Department of Biochemistry (Head – Prof. E. F. Shamrai) of the Kiev Medical Institute

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 70-74, January, 1960

Original article submitted November 22, 1958

The isolation of the individual vitamins and the study of their specific action on animals were the main tasks of research workers in the early period of development of vitaminology. At the present time, when vitamins are widely used in medical practice for therapeutic purposes, the question of the functional interrelationship between the members of this group of biologically active substances is one of particular urgency.

There are reports in the literature of metabolic synergism and metabolic antagonism between certain vitamins, and in particular, metabolic synergism has been established between vitamins C and P [1-4, 9, 10, 12, 16, 20].

In our laboratory we have synthesized the preparation "galascorbin", a complex compound of the sodium salts of ascorbic and gallic acids. Because of the presence of tannins in the composition of galascorbin, it possesses vitamin P as well as vitamin C activity. This preparation diminishes the permeability of capillaries, increases the resistance of animals to a rarefied atmosphere, and promotes the accumulation of ascorbic acid in the organs and tissues of guinea pigs under treatment for scurvy [9, 11, 17].

A series of investigations [1, 9, 13, 14, 15] has demonstrated the beneficial effect of galascorbin on wound healing. When injected parenterally, it stimulates repair processes in damaged tissues [8, 18, 19]. Since vitamin B_1 is directly concerned in the carbohydrate metabolism of tissues, some metabolic synergism between it and galascorbin was to be expected.

In order to test this hypothesis, we studied the influence of simultaneous injections of galascorbin and thiamin on the indices of the carbohydrate-phosphorus metabolism of traumatized tissues. We also tested the action of each preparation separately under the same conditions.

The object on which we carried out our comparative investigations was the muscle of a limb after the prolonged (for 6 hours) application of a tourniquet to arrest the blood flow.

As we have previously shown, application of a tourniquet to the limb of a rabbit for 6 hours is accompanied by the development of a severe atrophy of the muscles and by profound disturbances of their carbohydrate-phosphorus metabolism, the maximum changes being observed one month after trauma [6, 7].

METHOD

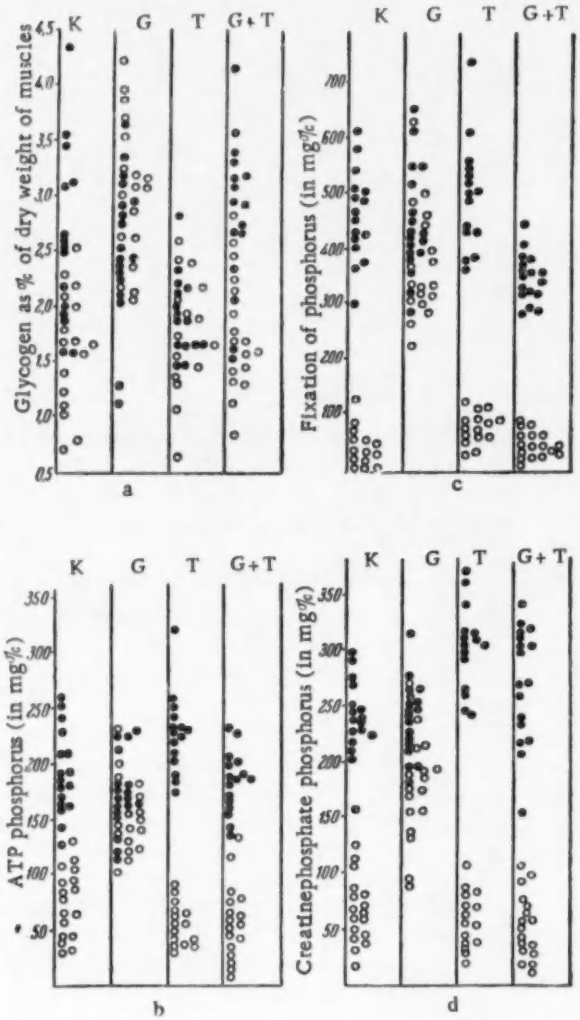
A tourniquet was applied to the middle third of the thigh of 80 rabbits, weighing 2.0-2.5 kg, for 6 hours, after which the animals were subdivided into 4 equal groups.

The first group acted as a control, and the animals in this group received a subcutaneous injection of 5 ml of physiological saline daily for one month. The animals of the second group received a subcutaneous injection of galascorbin, in a dose of 50 mg/kg body weight, made up in 5 ml of physiological saline, every day. The animals of the third group received daily subcutaneous injections of thiamin in a dose of 5 mg/kg body weight. Finally, the rabbits of the fourth group were injected with galascorbin and thiamin in the above doses, simultaneously.

One month after trauma, the gastrocnemius muscles from the injured and uninjured limbs were excised from the animals, under ether anesthesia. The muscles of the uninjured limb were used as a relative control. The content of glycogen, ATP, and creatine-phosphate and the phosphorylase activity in the muscles were determined by the generally accepted methods [5]. The results obtained were calculated per dry weight of muscle. In order to judge the degree of atrophy present, homonymous muscles of the two limbs were weighed (extensor digitorum longus).

RESULTS

In the animals of the control group, immediately after removal of the tourniquet, marked edema and paralysis of the traumatized limb were observed. After 4-5 days, the edema, as estimated by palpation, had disappeared, and on the 10th-12th day an appreciable diminution in the volume of the muscles of the injured limb was observed, by comparison with the muscles of



Biochemical indices of phosphorus metabolism in the muscles of rabbits. a) Glycogen; b) ATP; c) phosphorylase activity; d) creatine phosphate. Conventional signs: K) control; G) galascorbin; T) thiamin; G + T) galascorbin and thiamin. The black circles indicate the results for the uninjured limb, the clear rings those for the injured limb.

the intact limb. The atrophic changes progressed until the end of the observation. The motor function of the limb also remained disturbed one month after trauma, and in approximately one half of the cases, the paralysis of the limb was maintained.

An analogous clinical picture was observed in the animals receiving vitamin B_1 and also, contrary to our expectations, in the rabbits receiving galascorbin and thiamin simultaneously. Meanwhile, in the rabbits receiving galascorbin alone, the edema and subsequent reduction in volume of the traumatized muscles were much less pronounced than in the remaining groups, and the motor function of the limb was almost completely restored on the 15th-20th day after trauma.

Preliminary clinical observations alone were thus sufficient to suggest that vitamin B_1, in the doses which we used, not only did not stimulate the repair processes in the muscles undergoing compression ischemia, but completely abolished the therapeutic effect of galascorbin, so clearly demonstrated under these condtions.

Not to confine ourselves to clinical observations on the animals, we determined the main biochemical indices of carbohydrate – phosphorus metabolism in the muscles of the injured and uninjured limbs of the animals. The results of these investigations are shown in the form of a graph in the figure (the results of each examination of the muscles of the uninjured limb are indicated by black circles and those of the injured limb, by clear rings).

Despite the wide range of individual variations, in general characteristic of the components of muscle tissue which we were studying, the data in the figure illustrate the normalizing action of galascorbin on the carbohydrate – phosphorus metabolism of traumatized muscles and the absence of such action after administration of thiamin alone or thiamin in conjuction with galascorbin.

Since the individual variations in the levels of the components under study masked, to a certain extent, the results of the experiments, in each series of experiments we give the mean values and also the ratio between the indices of carbohydrate – phosphorus metabolism, as a percentage, in the injured and uninjured limbs (see Table).

It will be seen from the results of the investigation that the values obtained from biochemical analysis of the muscles fully agreed with the clinical observations.

In the animals of the control group one month after trauma, the weight of the muscles of the injured limb fell almost by one half, and this, together with the marked proliferation of connective tissue, was evidence of severe atrophy. The glycogen content fell by $\frac{1}{3}$, the ATP by $\frac{2}{3}$, the creatinephosphate by $\frac{3}{4}$, and the phosphorylase activity amounted to only 10% of the activity of the enzyme in the muscles of the uninjured limb. Similar results were obtained from investigations in animals receiving thiamin alone or thiamin and galascorbin simultaneously.

In contrast to this, in animals receiving galascorbin alone, the atrophy and the changes in carbohydrate-phosphorus metabolism of the injured muscles were less pronounced. The glycogen content, for example, even exceeded slightly that in the muscles of the uninjured limb. The ATP content reached 89%, whereas in the animals of the other groups it amounted to 24-38% of the ATP content in the muscles of the uninjured limb. The creatinephosphate content in the injured muscles was almost twice that in the animals not receiving galascorbin. The results of the determination of phos-

Weight and Indices of Carbohydrate–Phosphorus Metabolism of Gastrocnemius Muscles of Rabbits One Month After Application of Tourniquet (Mean Values)

Preparation injected	Weight of extensor digitorum longus, g			Glycogen, %			Phosphorylase activity, mg % of phosphorus combined during 2 hr incubation			ATP phosphorus, mg %			Creatinephosphate phosphorus		
	injured limb A	uninjured limb B	$\frac{A}{B} \cdot \%$	injured limb A	uninjured limb B	$\frac{A}{B} \cdot \%$	injured limb A	uninjured limb B	$\frac{A}{B} \cdot \%$	injured limbA	uninjured limb B	$\frac{A}{B} \cdot \%$	injured limb A	uninjured limb B	$\frac{A}{B} \cdot \%$
Physiological saline	1,22	2,16	56	1,67	2,55	65	51,2	459,8	11	68,5	176,7	38	62,2	238,0	26
Galascorbin	1,65	2,04	81	3,06	2,54	132	361,3	434,0	83	153,9	172,0	89	171,4	238,0	72
Thiamin	1,48	2,55	58	1,77	1,97	90	79,9	498,0	16	58,8	226,0	24	58,8	302,2	19
Thiamin and galascorbin	1,59	2,70	59	1,78	2,94	60	43,9	349,8	12	58,4	184,1	31	53,5	268,0	20

phorylase activity are particularly illuminating. Whereas in the animals of the other groups, the activity of the enzyme amounted to only 10-16% of that in the control muscles, in the group of rabbits receiving galascorbin, it reached 83%, i.e., it was much closer to normal.

It is a noteworthy fact that administration of galascorbin had no effect on the magnitude of the indices studied, in the muscles of the uninjured limb. In every case these values remained within normal limits. The preparation apparently had a selective action on injured tissue, in which it promoted the restoration of normal energy metabolism. It must be mentioned that, as we have repeatedly observed, the biological activity of vitamin P is clearly demonstrated when given to a patient with scurvy, but it cannot always be discovered when vitamin P preparations are given to healthy animals.

SUMMARY

We may thus conclude that the preparation "galascorbin", which possesses properties of vitamins C and P, exerts a definite stimulatory effect on repair processes in injured tissues, which gives prospects for the future clinical use of the drug on a wide scale.

Administration of vitamin B_1, in these experimental conditions, has no appreciable influence on the course of the atrophic process and on the biochemical indices of the energy metabolism of the injured muscles.

Vitamin B_1, injected simultaneously with galascorbin in proportions of 1 : 10 (5 mg thiamin and 50 mg galascorbin per kg body weight), abolishes the stimulating action of the galascorbin on the repair processes in the injured muscles.

This observation provides evidence of the necessity for further experimental investigation of the principles governing the use of vitamins in medical practice.

LITERATURE CITED

[1] Z. Zh. Gude, The Effect of Vitamin C in Combination with Certain Tannins on the Rate of Wound Healing [in Russian] Candidate's dissertation (Stanislav, 1954).

[2] K. L. Gurevich, Transactions of the Naval Medical Academy [in Russian] (Leningrad, 1947) 8, p. 151.

[3] N. A. Kovtunyak, Interaction between Vitamin C and the Polyphenols of the Wild Rose [in Russian] Candidate's dissertation (Stanislav, 1956).

[4] L. M. Krasnyanskii, Voprosy pitaniya 5, 92 (1958).

[5] A. L. Kursanov, V. N. Bukin, K. L. Plvolotskaya et al., Biokhimiya 15, 4, 337 (1958).*

[6] Ya. Z. Matusevich, Trudy Leningrad. san-gigien. med. inst. 14, 49 (1953).

[7] R. P. Nikolaev, K. L. Povolotskaya, and N. A. Vodolazskaya, Vitamins in Theory and Practice [in Russian] p. 111. (Moscow, 1953).

*Original Russian pagination. See C. B. Translation.

[8] A. Ya. Rozanov, Biokhimiya 23, 1, 66 (1958).•

[9] A. Ya. Rozanov, Voprosy pitaniya 17, 3, 43 (1958).

[10] D. L. Ferdman and E. F. Sopin, Practical Manual of Biological Chemistry [in Russian] (Moscow, 1957).

[11] T. V. Fetisova, The Biochemistry of Muscle [in Russian] (Kiev, 1954) p. 97.

[12] T. V. Fetisova, Vrachebnoe delo 2, 143 (1957).

[13] E. F. Shamrai, V. A. Nikonova, and U. A. Kuz'mins'ka, Ukrain. biokhim. zhur. 30, 1, 72 (1958).

[14] E. F. Shamrai, N. S. Verkhratskii, U. A. Kuz'minskaya et al., Collection of Abstracts of Scientific Papers of the Kiev Medical Institute, Period Ending 1955 [in Russian] (Kiev, 1957) p. 373.

[15] E. F. Shamrai and Z. Zh. Gude, Ukrain. biokhim. zhur. 24, 1, 102 (1952).

[16] E. F. Shamrai, Chemical Interaction and Biological Interrelationship between Ascorbic Acid and Certain Polyphenols [in Russian] Doctorate dissertation (Kiev – Stanislav, 1952).

[17] E. F. Shamrai and Z. Zh. Gude, Vrachebnoe delo 6, 654 (1957).

[18] E. F. Shamrai, Vrachebnoe delo 7, 715 (1957).

[19] E. F. Shamrai, Z. V. Karplyuk and Z. Zh. Gude, Ukrain. biokhim. zhur. 25, 1, 11 (1952).

[20] E. F. Shamrai and G. A. Gorchakova, Vrachebnoe delo 9, 815 (1953).

[21] B. I. Yanovskaya, Voprosy pitaniya 4, 14 (1953).

[22] L. Armentano, Ztschr. ges. exper. med. (1936) 97, p. 630.

[23] A. M. Ambrose and F. Deeds, J. Nutrition, 38, 305 (1936).

[24] A. Bentsath, S. Rusznyak, and A. Szent-Gyorgyi, Nature 138, 798 (1936). (November 7).

[25] V. Bruckner and A. Szent-Gyorgyi, Nature 138, (1936)

[26] H. Coterau, M. Gabe, E. Gero, and others, Nature 138, 161, 557 (1948).

[27] Di. F. Felice, Arch. sc. biol. 38, 113 (1954).

[28] S. Rusznyak, A. Szent-Gyorgyi, Nature 138, 27 (1936). (July 4).

[29] A. Szent-Gyorgyi, Ztschr. physiol. chimii, 255, 126 (1938).

•Original Russian pagination. See C. B. Translation.

ON THE MECHANISM OF ACTION OF GANGLIOPLEGICS ON PERIPHERAL VISCERO-VISCERAL REFLEXES

V. V. Zakusov and O. V. Ul'yanova

Institute of Pharmacology and Chemotherapy (Director – Active Member AMN SSSR V. V. Zakusov), AMN SSSR, Moscow

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 75-78, January, 1960

Original article submitted January 13, 1959

Our previous investigations [1] have shown that ganglionic blocking substances – tetraethylammonium (TEA), Pentamine (pentamethyldiethylenetriamine dibromoethylate), and hexamethonium – are able following total destruction of the central nervous system, to depress viscero-visceral reflexes, in particular those from the distal portion of the ileum to the proximal ileum and the jejunum, and from the urinary bladder to the colon, ileum, and jejunum. On the basis of these facts, and also in view of the fact that after the corresponding sympathetic ganglia are removed these reflexes are not observed, we advanced the interpretation that in this case ganglioplegic substances suppress local viscero-visceral reflexes. But the design of our experiments did not completely exclude the possibility that the observed phenomena develop on the basis of axon reflexes with preganglionic fibers participating.

To define this question more precisely we undertook new investigations in which the effect of ganglionic blocking agents on reflexes from the urinary bladder to the colon, ileum, and jejunum was studied following preliminary section of all preganglionic fibers of the inferior mesenteric ganglia. By this means degeneration of these fibers was produced, and consequently, the possibility of bringing about axon reflexes with the participation of preganglionic axons was eliminated.

METHODS AND EXPERIMENTAL RESULTS

The experiments were carried out on cats in which all the nerve fibers going to the inferior mesenteric ganglia from the sympathetic chain and the solar plexus were first (3-30 days previously) sectioned. This operation was carried out under ether anesthesia and aseptic conditions. Before the experiment the animals were anesthetized with ether, and the entire central nervous system was then destroyed. The animals' breathing was maintained artificially. The urinary bladder was stimulated by distending a rubber balloon which was inflated with air, usually under a pressure of 80 mm Hg. The pressure in the balloon was measured with a mercury manometer. The response of the colon, ileum, or jejunum was recorded with a water–air system which consisted of a rubber balloon, inserted into the lumen of the given segment of intestine, filled with water, and connected with a Marie capsule which recorded the contractions of the intestine on a kymograph. When the urinary bladder was distended, inhibition of the contractions of the colon, ileum, or jejunum occurred.

After degeneration of the preganglionic fibers of the inferior mesenteric ganglia, ganglionic blocking agents – TEA (1 mg/kg), Pentamine (2 mg/kg), and hexamethonium (1 mg/kg) – injected intravenously suppress reflexes from the urinary bladder to the colon, ileum, and jejunum (Fig. 1). These ganglioplegics produce this effect in doses at which they suppress the same reflexes without section of the preganglionic fibers, and also with the central nervous system intact. Thus the question of the possibility that the ganglioplegics suppress the indicated viscero-visceral reflexes with the participation of preganglionic fibers was settled by us in the negative.

In view of the fact that ganglionic blocking agents interfere with impulse transmission in ganglionic synapses, we could not assume that the excitation is transmitted from the urinary bladder to the colon, ileum, and jejunum in the manner of a postganglionic axon reflex. This would require that ganglionic blocking agents be able to exert an effect on the conduction of impulses along axons, for which there is no foundation.

To clarify the question of the effect of ganglioplegic substances on the peripheral component of local reflexes, we made a separate check of the transmission of excitation from postganglionic fibers to effector organs. In several experiments, we tested the state of impulse transmission from postganglionic fibers of the inferior mesenteric ganglion to the colon under the influence of ganglionic blocking agents. The postganglionic fibers of the inferior mesenteric ganglion were sectioned at the time of experiment, and were subjected to repetitive electrical stimulation (at a frequency of 30 cps) from an electronic generator. These experiments showed that TEA, Pentamine, and

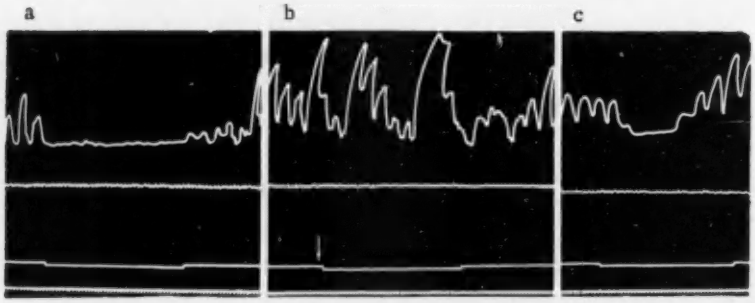


Fig. 1. Experiment 19 days after section of preganglionic fibers of the inferior mesenteric ganglion. Effect of Pentamine on reflex inhibition of contractions of small intestine following distention of urinary bladder. Interpretation of curves (top to bottom): contractions of intestine, cardiac contractions, stimulus marker, time marker (1 sec); a) before administration of Pentamine; b) 10 minutes after intravenous injection of 2 mg/kg Pentamine (↓); c) the same, after 1 hour and 45 minutes.

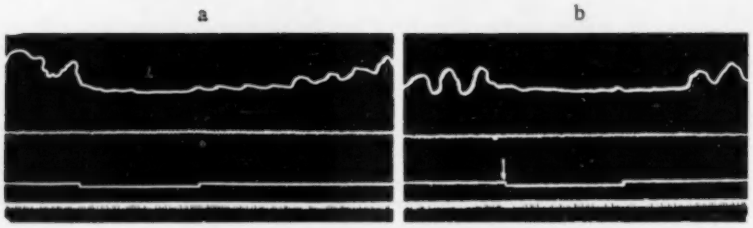


Fig. 2. Effect of hexamethonium on impulse transmission from postganglionic fibers of the inferior mesenteric ganglion to colon.
Curves (top to bottom): contractions of intestine, cardiac contractions, stimulus marker, time marker (1 sec); a) before administration of hexamethonium b) 15 minutes after intravenous injection of 2 mg/kg of hexamethonium (↓).

hexamethonium injected intravenously in doses at which local viscero-visceral reflexes are suppressed have no effect on the transmission of excitation from postganglionic fibers of the inferior mesenteric ganglion to the colon (Fig. 2). Consequently, the suppression of viscero-visceral reflexes by ganglionic blocking agents, observed by us, does not depend on any changes in impulse transmission in the peripheral component of these reflexes.

Thus ganglioplegic substances suppress the transmission of excitation from the urinary bladder to the intestine by interfering with synaptic transmission of impulses from afferent pathways to efferent ones – in the inferior mesenteric ganglia. In this case, "afferent pathways" must be understood to mean Dogiel's cells of the second type.

By way of developing this concept we carried out investigations of still another type. The effect of ganglioplegic substances on local reflexes was studied in experiments according to the classical scheme of N. M. Sokovnin [3] as modified by I. P. Razenkov [2], i.e., the effect of these substances on reflex contractions of the urinary bladder in response to stimulation of the central end of a sectioned hypogastric nerve was studied after preliminary section of all preganglionic fibers of the inferior mesenteric ganglia.

The experiments of this series, like the previous ones, were carried out on cats. From 1 to 30 days before the experiment, all fibers going to the inferior mesenteric ganglia from the sympathetic chain and the solar plexus were sectioned under aseptic conditions with the animals under nembutal anesthesia. By this means, degeneration of the preganglionic fibers of the inferior mesenteric ganglia was brought about.

Before the experiment, the animals were anesthetized with urethane or ether. After the vagus nerves were sectioned in the neck, the entire central nervous system was destroyed. The animal's breathing was maintained artificially. The abdominal cavity was then opened, and one of the hypogastric nerves was sectioned.

The central end of this nerve was repetitively stimulated with sinusoidal stimuli (frequency 20 cps, amplitude 0.5-2 V) from an electronic generator.

The response to stimulation of the central end of the hypogastric nerve appeared as a contraction of the urinary bladder. These contractions were recorded on a kymograph by a mechanographic air–water system consisting of a rubber balloon filled with water

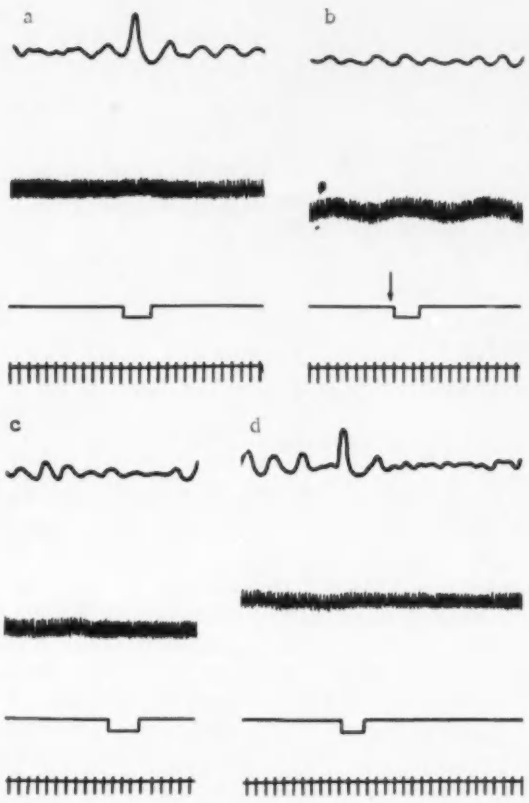


Fig. 3. Experiment 12 days after section of preganglionic fibers of the inferior mesenteric ganglion. Effect of hexamethonium on reflex contraction of urinary bladder upon stimulation of central end of sectioned hypogastric nerve. Interpretation of curves (top to bottom): contractions of urinary bladder, cardiac contractions, stimulus marker, time marker (1 sec); a) before administration of hexamethonium; b) 5 minutes after intravenous injection of 2 mg/kg hexamethonium (↓); c) the same, after 20 minutes; d) the same, after 2 hours.

and inserted into the urinary bladder through an incision in the apex, or a catheter inserted through the urethra.

It was found that even after degeneration of the preganglionic fibers of the inferior mesenteric ganglia, TEA (1–2 mg/kg), Pentamine (2–3 mg/kg), and hexamethonium (1.5–2 mg/kg) injected intravenously suppress contractions of the urinary bladder in response to stimulation of the central end of the sectioned hypogastric nerve (Fig. 3). Thus ganglioplegic substances suppress many peripheral viscero-visceral reflexes after degeneration of preganglionic fibers. When we consider that these substances have no effect on the transmission of excitation from postganglionic fibers to effector organs, we can only conclude that ganglionic blocking agents suppress the transmission of excitation from afferent to efferent pathways in the autonomic ganglia.

SUMMARY

The authors investigated the effect of tetraethylammonium, Pentamine, and hexamethonium on reflexes from the urinary bladder to the colon, ileum, and jejunum, and on the reflex contractions of the urinary bladder following stimulation of the central end of the sectioned hypogastric nerve, after preliminary section (3-30 days previously) of all the preganglionic fibers of the inferior mesenteric ganglion. This was done to ascertain the mechanism of action of the ganglioplegics on peripheral viscero-visceral reflexes. It was found that these substances continued to depress the reflexes mentioned after degeneration of the preganglionic axons of the inferior mesenteric ganglia.

Thus the data obtained demonstrate that the ganglionic blocking agents depress local reflexes, thus inhibiting the transmission of excitation in autonomic ganglia from afferent to efferent neurons.

LITERATURE CITED

[1] V. V. Zakusov and O. V. Ul'yanova, Farmakol. i toksikol. 21, 2, 3-8 (1958).

[2] I. P. Razenkov, Zhurn. eksperim. biol. i med. 3, 66 (1926).

[3] N. M. Sokovnin, Izvestiya i uchenye zapiski kazanskogo universiteta 44, 5, 1243 (1877).

CENTRAL ANTAGONISM BETWEEN GANGLIOLYTIC AGENTS AND NEOSTIGMINE METHYLSULFATE

M. L. Tarakhovskii

Department of Pharmacology (Head – Prof. S. P. Zakrividoroga) of the Chernovits Medical Institute and the Division of Pharmacology (Head – Active Member AMN SSSR S. V. Anichkov) of the Institute of Experimental Medicine, AMN SSSR, Leningrad

(Presented by Active Member AMN SSSR S. V. Anichkov)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 78-83, January, 1960

Original article submitted January 28, 1959

The study of the interrelations between cholinergic substances and anticholinesterase preparations has been advanced by the demonstration of several new factors in the physiology and pharmacology of the central nervous system. Data have been obtained which confirm the cholinergic nature of the functions of the mesodiencephalic reticular formation [6, 9, 10], the presence of choline-reactive structures in the cerebral cortex has been established [4], and the location and character of the central action of acetylcholine and its antagonists – atropine, scopolamine, etc. – have been defined more precisely.

At the same time it should be remembered that the overwhelming majority of works of this kind are associated with the use of "tertiary" cholinolytics. It appears that the explanation of this is to be sought in the fact that the central action is considerably weakened in passing from the "tertiary" nitrogen derivatives to the "quaternary" ones, since the penetration of the latter into the central nervous system is impeded. Nevertheless realization of this fact should not limit research on the central mechanisms in the case of "quaternary" gangliolytics, which certainly have an effect on the central nervous system [1, 2, 5, 7].

The purpose of the present investigation was to study the interaction between the "quaternary" gangliolytics and neostigmine methylsulfate, with the help of methods that reflect the functional state of the central nervous system.

METHODS

For determination of functional changes in the central nervous system, recording of biopotentials of the cortex and subcortical regions was employed. Action potentials from the frontal lobe of the cortex were picked up with unipolar leads, carried to a balanced amplifier of a cathode-ray oscilloscope, and recorded photographically with a motion picture camera. Unipolar recording of action potentials of the subcortical region was carried out on decerebrate cats. The active electrode was inserted in the direction of the floor of the fourth ventricle, as determined by brain section. In the second series of experiments, the latent period of a flexor reflex was determined by the method of V. V. Zakusov [3]. Cutaneous stimulation in the femoral region was brought about with the help of needle-shaped electrodes. The current from an electronic rectifier plugged into the line was used as a stimulus. The stimulus intensity was determined from the voltage. Time was indicated automatically by a type EMS-4 millisecond clock. Various derivatives of the di-iodide of hexamethylene-bis-(trimethylammonium), substituted with cations and anions, were used as gangliolytics. The experiments were performed on 26 rabbits and 14 cats.

RESULTS

Changes in the bioelectrical activity of the brain occurring under the influence of these gangliolytics were used as indicators of functional shifts in the central nervous system. In rabbits, these changes were usually of a phasic character. Upon intravenous administration of gangliolytics in doses of 0.5-5 mg/kg obliteration of the slow waves and increased amplitude and frequency of the fast waves (to 55-75 μV and 20-25 per second) were initially noted. In the second phase, which came on 5-45 minutes after injection of the substance, a considerable reduction of the bioelectrical activity of the frontal lobe of the cortex was seen, and the electroencephalogram assumed a flattened character. Changes in the bioelectrical activity of the cerebral cortex were recorded for 1-4 hours from the time of injection of the gangliolytic.

The bioelectric activity of the hypothalamic region of the cat brain also underwent substantial changes under the influence of these preparations. Both intravenous injection and injection directly into the cavity

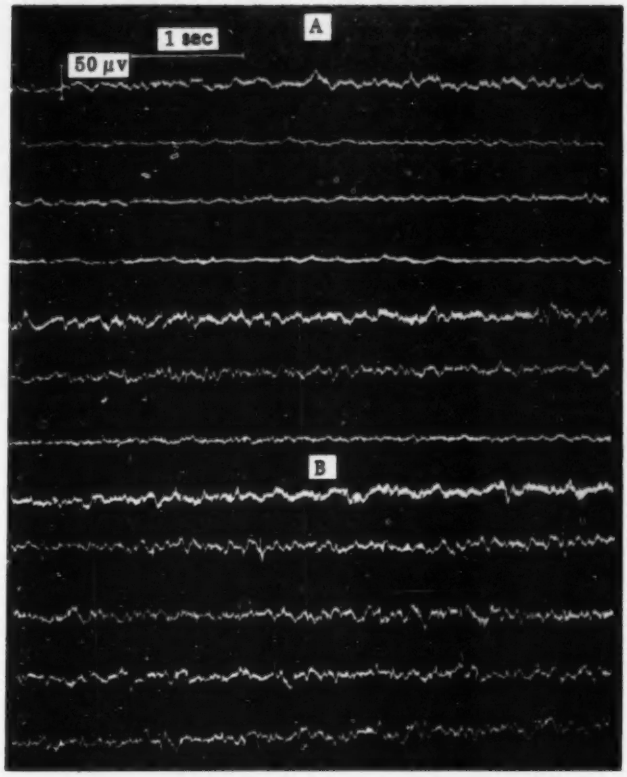


Fig. 1. Effect of gangliolytics and neostigmine methylsulfate on bioelectrical activity of the cerebral cortex of the rabbit. Record of action potentials of frontal lobe. A) Effect of di-p-amino-benzoyl salt of hexamethylene-bis-ethyldimethylammonium in a dose of 5 mg/kg. 1) Normal; 2,3,4,5,6, 7) 5,10,20,30,60 and 90 minutes after injection of the preparation. B) Effect of the same preparation against a background of neostigmine methyl-sulfate (0.2 mg/kg intravenously). 8) normal; 9,10,11, 12) 5, 10,30, and 60 minutes after injection of gangliolytic.

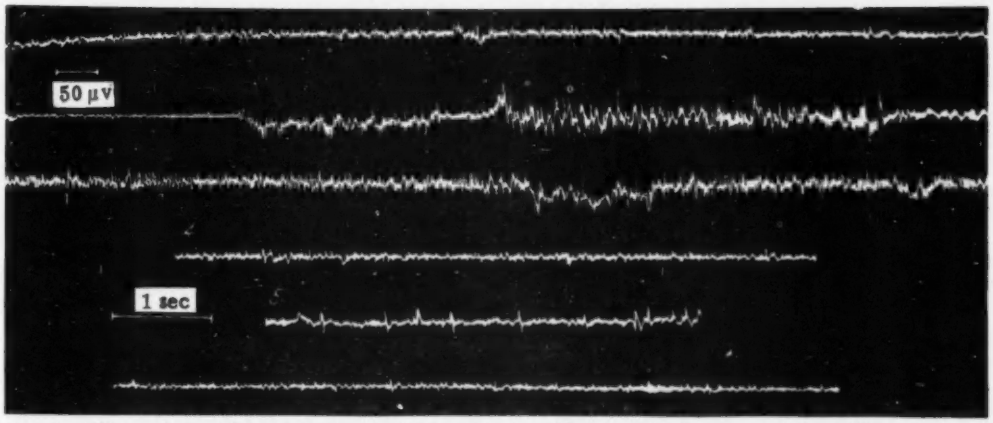


Fig. 2. Effect of gangliolytics and neostigmine methysulfate on bioelectric activity of hypothalamic region of brain cat. 1) Normal; 2, 3, 4-3, 10 and 30 minutes after injection of 1 mg/kg of di-pyri-dine-β-carboxyl salt of hexamethylene-bis-triethylammonium into cavity of 4th ventricle; 5, 6) 3 and 10 minutes after injection of the same preparation against a background of neostigmine methyl-sulfate (40 γ/kg into the cavity of 4th ventricle).

of the 4th ventricle were accompanied by depression of bioelectrical activity, and reduction of the amplitude of all components of the electroencephalogram. In a number of experiments in which relatively small doses of gangliolytic were injected into the cavity of the 4th ventricle (0.1-1 mg/kg) synchronous, peak-shaped waves with a frequency of 3-40 per second and an amplitude of 30-60 μV were seen to appear.

It was established that the character of the anion had a substantial effect on the duration and depth of the functional shifts in the central nervous system, as determined by the electroencephalogram.

The salts of "quaternary" nitrogen derivatives substituted with anions of nitrogen-containing cyclic radicals – β-pyridine-carboxyl, p-nitrobenzoyl, p-aminobenzoyl – were more active in this respect than their halogen homologs.

The data obtained served as a basis for conducting experiments in which the effect of neostigmine methylsulfate on changes on the electroencephalogram produced by gangliolytics was studied. It was first established that neostigmine methylsulfate administered intravenously in doses of 0.05-0.1 mg/kg produced no substantial changes in the bioelectrical activity of the cortex and subcortical regions. We also found corresponding data in the literature [6, 8].

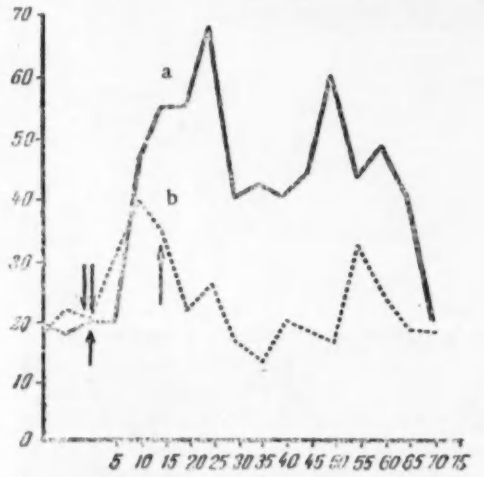


Fig. 3. Effect of gangliolytics and neostigmine methylsulfate on the latent period of flexor reflex of the rabbit. a) Following injection of dipyridine-β-carboxyl salt of hexamethylene-bistriethylammonium (1 mg/kg intravenously); b) following injection of the same preparation against a background of neostigmine methylsulfate (0.05 mg/kg intravenously). Arrows indicate the moment of injection of gangliolytic (↑) and neostigmine methylsulfate (⇓). Along abscissa – reflex time in milliseconds; along ordinate – duration of experiment in minutes.

In various versions of the experiment – with preliminary, simultaneous, and subsequent administration of neostigmine methylsulfate – it was established that this compound significantly reverses the bioelectrical reaction to a gangliolytic. The slow variations, with a frequency around 3-4 per second and amplitude around 50-60 μv, remained even after administration of gangliolytic; the rhythm of the oscillations with moderate frequency and amplitude (of the order of 10-30 μv and 10-20 per second) was somewhat accelerated, but did not reach the control levels in a single experiment. The second phase of action of the gangliolytic – depression of the bioelectrical activity of the cortex – was completely absent (Fig. 1).

The antagonistic effect of neostigmine methylsulfate on the central actions of the gangliolytic also appeared in experiments on cats in which injections were made into the 4th ventricle. It should be noted that neostigmine methylsulfate was active not only against the inhibitory effects of these substances on bioelectrical activity of the hypothalamic region of the brain, but also towards the excitatory action which was observed in several instances when comparatively small doses of gangliolytics were injected into the cavity of the 4th ventricle. The large amplitude and rapid peak-shaped rhythm characterizing the excitation of bioelectrical activity of the subcortical region of the brain was absent following a single injection of neostigmine methyl sulfate and gangliolytic (Fig. 2). Repeated injections of small doses of gangliolytic into the cavity of the 4th ventricle of the same animal, made for purposes of control, showed that when the interval between injections is not less than an hour, the excitatory action of the preparations of this series remains, as determined by the electroencephalogram.

The central antagonism between these substances also was manifested in suppression of the motor reaction which occurred in several experiments when the ganglionic blocking agent was injected into the cavity of the 4th ventricle.

In the second series of experiments we studied the effect of neostigmine methylsulfate on the changes in the latent period of the flexor reflex observed in rabbits following the administration of gangliolytics. In control experiments, it was established that substances of this group injected intravenously in doses of 0.5-5 mg/kg produce a distinct slowing of the conduction of impulses through the synaptic apparatus of the spinal cord. The latent period of the reflex was 18-34 milliseconds in various rabbits. When the ganglioplegic was injected, an increase in the latent period ensued as early as 5-10 minutes after injection and remained for from 30 minutes to 4 hours. The latent period of the flexor reflex increased in various experiments to 50-190 milliseconds, which was 2-4 times longer than the original values.

Preliminary (10-15 minutes previously) intravenous injection of neostigmine methylsulfate in a dose of 0.05 mg/kg, which somewhat prolonged the latent period of

the reflex in the majority of experiments, reversed the similar effect of the gangliolytics.

In those experiments in which the effect of gangliolytics was still seen, it was considerably weaker and briefer against a background of neostigmine methylsulfate. The mean values shown in Fig. 3, calculated on the basis of 5 experiments with uniform characteristics, show the antagonistic action of gangliolytics and neostigmine methylsulfate on the latent period of the flexor reflex in the rabbit (Fig. 3).

The antagonistic effect of these substances was also displayed in the fact that the fasciculations of the muscles of the trunk and extremities and the quickened breathing seen in rabbits after injection of neostigmine methylsulfate were suppressed by gangliolytics.

The data presented confirm the presence of a central antagonism between "quaternary" gangliolytics and neostigmine methylsulfate. Such interrelations appear at various functional levels of the central nervous system – in the cortex, the subcortical region, and the spinal cord. These facts give evidence of the cholinolytic nature of the central action of ganglionic blocking agents – the derivatives of "quaternary" ammonium bases.

SUMMARY

The interrelations between gangliolytics – derivatives of the quaternary ammonium bases – and neostigmine methylsulfate were investigated by methods reflecting the functional state of the central nervous system. As shown by experiments in which cerebral biopotentials were recorded in rabbits and cats, neostigmine methylsulfate considerably reverses the bioelectrical reaction to gangliolytics. The antagonism between these substances is also demonstrated by the fact that neostigmine methylsulfate reverses the retardation of synaptic impulse transmission along the spinal cord caused by gangliolytics. The present data point to the fact that the central action of the series of compounds investigated is cholinergic in nature.

LITERATURE CITED

[1] B. B. Voznesenskii, Farmakol. i toksikol. 21, 4, 7 (1958).*

[2] P. P. Denisenko, Gangliolytics and Substances that Block Neuromuscular Junctions (Leningrad, 1958) p. 21 [in Russian].

[3] V. V. Zakusov, Fiziol. zhurn. SSSR 23, 2, 276 (1937).

[4] M. Ya. Mikhel'son, E. K. Rozhkova, and N. V. Savateev, Byull. eksperim. biol. i med. 37, 2, 7 (1954).

[5] M. L. Tarakhovskii, Farmakol. i toksikol. 20, 1, 33 (1957).*

[6] P. B. Bradley and J. Elkes, J. Physiol., 1953, v. 120, Proceeding p. 14.

[7] A. S. Doutas and M. J. Nickerson, J. Pharmacol. a. Exper. Therap., 1957, v. 120, p. 147.

[8] W. H. Funderburk and T. J. Case, Electroencephal. a. Clin. Neurophysiol., 1951, v. 3, p. 213.

[9] F. Rinaldi and H. E. Himwich, Arch. Neurol. a. Psychiat., 1955, v. 73, p. 387, p. 396.

[10] W. C. Wescoe, R. E. Green, B. P. McNamara, and others, J. Pharmacol. a. Exper. Therap., 1948, v. 92, p. 63.

*Original Russian pagination. See C. B. Translation.

EFFECT OF GLUTAMIC ACID ON CHOLINERGIC EXCITATION IN CENTRAL AND PERIPHERAL SYNAPSES

P. E. Dyablova

Department of Pharmacology (Head – Corresponding Member AMN SSSR Prof. V. M. Karasik) of the Leningrad Pediatric Medical Institute (Director – N. T. Shutova)

(Presented by Active Member AMN SSSR S. V. Anichkov)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 83-86, January, 1960

Original article submitted January 27, 1959

One of the ways ammonia is detoxified in the animal organism is by combining with glutamic acid, with the formation of glutamine. This is why preliminary introduction of glutamic acid into the organism of rabbits, rats, and dogs prevents the excitation of the central nervous system caused by ammonium salts [3, 4, 14].

It is well known that in any excitation of the central nervous system the concentration of ammonia, which is liberated from glutamine and adenylic acid, increases in the brain tissue. For this reason various authors have tested the effect of glutamic acid on convulsions produced by administration not only of ammonium salts but also of other substances. In experiments on mice, rats, cats, rabbits, and monkeys, however, it was ineffective in preventing convulsions occurring under the influence of pentylenetetrazol (Corazol), and electroconvulsions [11]. An insignificant anticonvulsive effect was found when glutamic acid was tested in experiments on mice and rats subjected to the action of elevated oxygen tension [5]. Nevertheless, the clinical literature of the last fifteen years contains a large number of works devoted to the administration of glutamic acid in small epileptic seizures [1, 2, 13, etc.]. An antiepileptic effect, however, is seen only when glutamic acid is used in combination with anticonvulsive substances (phenobarbital, bromides, etc). Observations have been described in which agents with an anticonvulsive action failed to give a positive result in the treatment of patients with small epileptic seizures; on the other hand, the combination of these agents with glutamic acid proved effective. Many authors explain this therapeutic effect of glutamic acid by its capacity, established previously, to combine with ammonia.

In view of the hypothesis that epileptic seizures are cholinergic in nature [15, etc.], we undertook a study of the influence of glutamic acid on the effects of neostigmine methylsulfate, which promotes cholinergic excitation by depressing the enzymatic hydrolysis of acetylcholine.

METHODS AND RESULTS

In the first series of experiments we studied the effect of glutamic acid on neostigmine methylsulfate intoxication in white mice. The experiments were carried out on 100 adult male animals weighing 16 – 22 g. The substances under investigation were injected subcutaneously.

Neostigmine methylsulfate in a dose of 0.2 γ per g weight of mouse produced obvious muscular fasciculation in all the animals. When neostigmine methylsulfate was injected in a dose of 0.3 γ per g weight, convulsions occurred, terminating in death in 62.5% of the cases. When this difference had been established, the effect of preliminary injection of glutamic acid on the muscular fasciculation caused by neostigmine methylsulfate was ascertained: 10 mice were injected with glutamic acid (in a dose of 300 γ per g weight) 10 minutes before injection of neostigmine methylsulfate (in a dose of 0.2 γ per g weight). In this dose, glutamic acid by itself caused some limitation of activity in the mice. Ten control animals received no glutamic acid. Glutamic acid had essentially no effect on the muscular fasciculations: It delayed the time of appearance only insignificantly, slightly reduced their intensity, and had no effect on the duration.

In subsequent experiments, 40 mice were injected with glutamic acid (300 γ per g weight) 10 minutes before injection of neostigmine methylsulfate (0.3 γ per g weight); 40 control animals were injected with neostigmine methylsulfate only. For comparison, the effect of glutamic acid on pentylenetetrazol-induced convulsions in white mice was tested (according to published data, convulsions of this type are not in the main of cholinergic origin [15]). This series of experiments was carried out on 80 adult male animals weighing 16 – 22 g; 40 mice were injected with glutamic acid in the same dose as in the experiments with neostigmine methylsulfate, 10 minutes before injection of

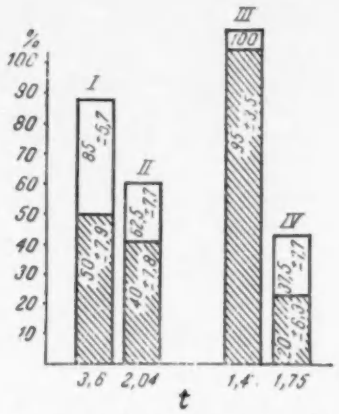


Fig. 1. Effect of glutamic acid on the course and outcome of intoxication with neostigmine methylsulfate and pentylenetetrazol. I, II) Experiments with neostigmine methylsulfate; III, IV) experiments with pentylenetetrazol.

pentylenetetrazol (80 γ per g weight). Control mice received only pentylenetetrazol (80 γ per g weight).

In experiments with neostigmine methylsulfate, preliminary administration of glutamic acid reduced the number of animals in which convulsions appeared (from 85% to 50%), and the number of animals that died (from 62.5% to 40%). The same dose of glutamic acid, tested in experiments with pentylenetetrazol, did not have a clear-cut anticonvulsive effect (100% of the animals showed convulsions in the control, and 95% in the experimental group), but reduced the number of deaths to some extent (from 37.5% to 20%). Thus the inhibiting effect of glutamic acid was more clearly apparent in convulsions caused by neostigmine methylsulfate, which are evidently cholinergic in nature.

Figure 1 shows the results of experiments with neostigmine methylsulfate and pentylenetetrazol. The number of mice in which convulsions developed (see Fig. 1, I and III), and the number of animals that died (see Fig. 1, II and IV) are expressed in percentages with the error calculated according to the formula:

$$m = \pm \sqrt{\frac{v(100 - v)}{n}},$$

where m is the error of the index; v is the index (percent of animals in which convulsions occurred, or percent of animals that died); and n is the number of animals.

The percent of animals in which convulsions appeared, and the percent of animals that died, in the control experiments, are indicated in Fig. 1 in the upper, unshaded part of the column; and the percent of mice in which convulsions occurred, and the percent of animals that died, after preliminary injection of glutamic acid, are indicated in the shaded portion of the column. Under the columns are shown the reliability criteria t, which are obtained by dividing the differences between the indices in percent by the root-mean-squares of the errors. Whereas the positive effect of glutamic acid on the course and outcome of neostigmine methylsulfate intoxication was statistically reliable (t = 3.6 and 2.04), the corresponding effect on the course and outcome of pentylenetetrazol intoxication was not reliable (t = 1.4 and 1.75); for the number of animals that we used in the experiment, the value of t for reliability should be no less than 2.02.

In a second series of experiments we studied the effect of glutamic acid, as well as that of glutamine and asparagine, on the contractile activity of the isolated rectus abdominis muscle of the frog.

We described this contractile activity in 1948 [6]. It was first found in the rectus abdominis muscle and the gastrocnemius muscle of the frog, and later also in the sartorius muscle [10]. Its occurrence is explained by enhancement of the effect of acetylcholine: In the isolated muscle acetylcholine is liberated spontaneously in amounts insufficient to cause contractions; but under the influence of neostigmine methylsulfate, which depresses cholinesterase and apparently also facilitates the response to acetylcholine, contractile

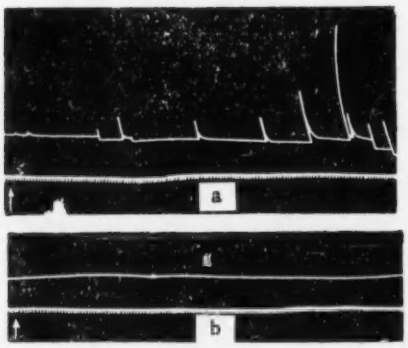


Fig. 2. Contractions of frog rectus abdominis muscle (a) under the influence of neostigmine methylsulfate (1: 250,000). The segment of the myogram corresponds to 30 minutes (during this time the kymograph was stopped repeatedly, for a total of 19 minutes). The arrows indicate the addition of neostigmine methylsulfate Time marker (5 seconds). Myogram of the same muscle (b) after the neostigmine methylsulfate had been repeatedly washed out and replaced with Ringer's solution containing glutamic acid (1: 1000), with subsequent addition of neostigmine methylsulfate (1: 250,000). Segment of myogram corresponds to 30 minutes (during this time the kymograph was repeatedly stopped, for a total of 20 minutes). Designations and time marker are the same as in Fig. 2a.

activity develops which is accordingly of a cholinergic nature [6]. The data obtained by us have been reproduced by N. A. Kozlova and M. Ya. Mikhel'son [9] and by G. A. Panosyan [10], who have adopted our interpretation of the neostigmine methylsulfate effect.

The isolated frog rectus abdominis muscle was subjected to the action of the substances being tested, 1-2 hours after isolation. Neostigmine methylsulfate was tested in concentrations of 1: 200,000–300,000. The contractions that appeared were recorded on a kymograph for 30-40 minutes, after which solutions of glutamic acid, glutamine, or asparagine were added. (The glutamic acid solutions were neutralized with sodium bicarbonate; solutions of the other amino acids gave a neutral reaction). In another version of the experiment, after recording of the neostigmine methylsulfate contractions the neostigmine methylsulfate was rinsed out and subsequently replaced by Ringer's solution containing glutamic acid, or asparagine, and after 30 minutes neostigmine methylsulfate was again added in the concentration tested earlier.

According to the data of these experiments, glutamic acid (in concentrations of 1: 10,000 and above) suppresses the neostigmine methylsulfate contractions of muscle (Fig. 2a and b). Glutamine and asparagine at higher concentrations (1: 500-1000) inhibit neostigmine methylsulfate contractions, but at lower concentrations (1: 10,000 -20,000) they enhanced the neostigmine methylsulfate contractions in 4 of the 7 tests. Thus glutamic acid also showed a capacity to disturb cholinergic excitation in experiments on the isolated muscle. Although this effect was also demonstrated under the influence of glutamine and asparagine, glutamic acid produces it in solutions at least 5-10 times more dilute (since the molecular weights of glutamic acid, glutamine, and asparagine are almost exactly equal, the molar ratios are the same).

The experimental material obtained indicates that glutamic acid has the capacity to affect one of the steps in cholinergic excitation. This is displayed more distinctly in the neuromuscular junctions (suppression of contractile activity of muscles occurring under the influence of neostigmine methylsulfate); a less marked effect appears in the central synapses (anticonvulsive action). Presumably such an effect of glutamic acid can be explained by its action on the synthesis or on the binding of acetylcholine. The former idea is supported by the data of D. Nachmansohn and his collaborators [12], who have established that sufficiently high concentrations of glutamic acid are able to inhibit acetylcholine synthesis (in contrast to lower concentrations, which favor this synthesis). The second interpretation conflicts with results obtained on brain tissue sections [16]. According to the experiments of Tower and Elliott [16], in epilepsy, hypoxia, and poisoning with the convulsive poison methionine sulfoximine, the ability of brain tissue to bind acetylcholine is disturbed; glutamine and asparagine eliminate this disturbance, but glutamic acid does not have such an effect. It should be said, however, that the data of Tower and Elliott, obtained with sections, can not be unconditionally applied to processes occurring in the isolated muscle or in the intact organism. For this reason, we do not believe that the second interpretation can be rejected. It is possible that glutamic acid has an effect on the Krebs cycle, which is responsible not only for acetylcholine synthesis, but also for binding of acetylcholine. Finally, glutamic acid might play a role in our experiments as a substance that binds ammonia. Although the fact that ammonia is formed in convulsions cannot be denied, its importance in their production is apparently slight.

SUMMARY

It has been established experimentally that glutamic acid depresses the contractile activity of frog rectus abdominis muscle under the influence of neostigmine methylsulfate, and reduces the percentage of white mice suffering convulsions or death after neostigmine methylsulfate poisoning. The author suggests that these effects of glutamic acid may be explained by its action upon the synthesis or binding of acetylcholine.

LITERATURE CITED

[1] A. L. Andreev, Therapeutic Administration of Amino Acids in Nervous and Mental Diseases [in Russian] (Moscow, 1957).

[2] A. L. Andreev and E. K. Khalizeva, Zhur. nevropatol. i psikhiat. 55, 5, 359 (1955).

[3] N. V. Veselkin and B. G. Gordon, Doklady akad. nauk SSSR 107, 2, 333 (1956).

[4] E. A. Vladimirova, Byull. éksperim. biol. i med. 11, 345 (1950).

[5] Z. S. Gershenovich and A. A. Krichevskaya, Biokhimiya 17, 6, 684 (1952).

[6] P. E. Dyablova, Byull. eksperim. biol. i med. 25, 6, 461 (1948).

[7] P. E. Dyablova, Farmakol. i toksikol. 2, 45, (1950).

[8] P. E. Dyablova, Fiziol. Zhur. SSSR 32, 3, 266 (1957).

[9] N. A. Kozlova and M. Ya. Mikhel'son, Fiziol. zhur. SSSR 3, 362 (1951).

[10] G. A. Panosyan, Izvest. akad. nauk Armyanskoi SSR, biol. i sel'khoz. nauki 9, 11, 35 (1956).

[11] L. S. Goodman, E. A. Swinyard, and J. E. Toman, Arch. Neurol and Psychiat, 56, 20 (1946).

[12] S. R. Korey, R. Braganza, and D. Nachmansohn, J. Biol. Chem. 189, 705 (1951).

[13] J. C. Price, H. Waelsch and T. J. Putnam, J. A.M.A. 122, 1153 (1942).

[14] M. R. Sapirstein, Proc. Soc. Exper. Biol and Med. 52, 334 (1943).

[15] W. E. Stone, Am. J. Phys. Med. 36, 222 (1957).

[16] D. B. Tower and K. A. C. Elliott, J. Appl. Physiol. 5, 375, (1953).

CHANGES IN THE ANTIGENIC PROPERTIES OF THE BLOOD SERUM AFTER PARENTERAL INJECTION OF NONPROTEIN SUBSTANCES INTO AN ANIMAL

A. I. Nikolaev

Tashkent Pharmaceutical Institute (Director – Docent M. A. Azizov) and the Department of Microbiology (Head – Prof. P. F. Samsonov) of the Tashkent Medical Institute

(Presented by Active Member V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 87-90, January, 1960

Original article submitted March 18, 1958

We have shown [3] by the method of immunization, sensitization, desensitization, and anaphylaxis, that the antigenic function is a property of substances of protein nature only. The formation of antibodies to substances of nonprotein nature is explained by the formation of complexes by the substances injected and the animal's own proteins, so that the proteins are denatured and converted into foreign protein [1].

Denaturation changes in the serum proteins of horses hyperimmunized with antitoxins are shown, in particular, by the fact that diphtheria, tetanus, and botulinus antisera are extremely resistant to heat [2, 4]. Hypotheses concerning the changes in the antigenic properties of the proteins of the body by the action of injected compounds have not yet, however, received experimental confirmation.

In the present investigation our aim was to bring to light denaturation changes in the serum proteins and in their antigenic properties.

METHOD AND RESULTS

In order to dectect denaturation changes in the serum proteins after injection of various substances into animals, we used F. S. Okolov's method [4]. Rabbits were immunized with 1% solutions of horse serum sulfanylazoglobulin, sulfanylazostarch, sulfanylazosalicylic acid and a mixture of this with serum, starch solution, a 5% solution of sulfanylazosalicylic acid, and normal horse serum (control). On the day of the last injection, the animals' serum was tested for its thermal stability, and on the 8th day its antibody content was determined by the precipitation method. The thermal stability of the serum proteins was determined in a volume of 1 ml, at 61-62°. Heating was contined until the serum was completely gelatinized.

The results of the experiments are shown in Table 1.

It will be seen from Table 1 that solutions of starch and sulfanylazosalicyclic acid, when given by the usual methods of immunization, did not bring about the appearance of antibodies and did not affect the thermal stability of the serum proteins. A 5% solution of sulfanylazosalicylic acid sharply increased the thermal clotting time of the sera and led to the formation of precipitins (rabbits nos. 18, 19, and 20). An analogous phenomenon, although less pronounced, was observed after injecting the animals with sulfanylazoglobulin, sulfanylazostarch and a mixture of sulfanylazosalicylic acid with protein. In a control experiment, no changes were observed in the thermal clotting time.

This series of experiments thus demonstrated a link between the formation of precipitins to azo-compounds and the denaturation changes in the serum proteins of animals. No quantitative relationship could be found between the thermal clotting time and the antibody titer.

In the next series of experiments, wer tested the changes in the antigenic properties of the body proteins under the influence of sulfanylazostarch and sulfanylazosalicylic acid. In this case we took into consideration the fact that substances not possessing antigenic properties may desensitize animals for several hours [3, 5, 6], but true antigens for a much longer period (2-3 weeks). With regard to the substances under test, we found that [3] sulfanylazostarch desensitized guinea pigs for periods of up to 24 hours, but sulfanylazosalicylic acid only for up to ½ hour; a mixture of solutions of sulfanylazosalicylic acid and protein possessed a prolonged desensitizing action. It could be concluded from these results that sulfanylazosalicylic acid and sulfanylazostarch had no inherent antigenic function, but the immunological specificity of the proteins was modified under their influence. These observations paved the way for experiments to determine the changes in the antigenic properties of the proteins in the animal body.

In order to modify the antigenic properties of the serum proteins of animals the preparations were injected intravenously into rabbits in a dose of 10 ml of a 4% solution, over a period of 2 days. On the second day, 4 hours after injection, the serum was tested for the presence of proteins with modified antigenic properties, by

TABLE 1 Antibody Titer and Thermal Clotting Time of Immune Sera

Immune serum	Rabbit No.	Dilution of test antigen—human sulfanylazoglobulin						Normal horse serum	Thermal clotting time (in minutes)	
		1:10	1:20	1:40	1:80	1:160	1:320		before immunization	after immunization
Against 1% starch solution	1	0	0	0	0	0	0	0	125	140
	2	0	0	0	0	0	0	0	105	130
Against 1% solution of sulfanylazosalicylic acid	3	0	0	0	0	0	0	0	135	140
	4	0	0	0	0	0	0	0	140	135
	5	0	0	0	0	0	0	0	100	115
Against 5% sulfanylazosalicylic acid solution	18	++	+	0	0	0	0	0	100	245
	19	+++	++	+	0	0	0	0	125	260
	20	++	0	0	0	0	0	0	120	200
Against 1% sulfanylazosalicylic acid solution in 1% horse globulin solution	15	+++	++	+	0	0	0	++++	125	165
	16	+++	++	0	0	0	0	++++	120	170
	17	+++	+++	++	0	0	0	++++	110	130
Against 1% horse sulfanylazoglobulin solution	9	++++	++++	++++	+++	++	+	++++	100	205
	10	++++	++++	+++	++	+	0	++++	130	170
	11	++++	++++	++++	+++	+++	++	++++	95	165
Against 1% sulfanylazostarch solution	12	+++	++++	+++	++	0	0	0	120	215
	13	+++	+++	+	0	0	0	0	90	235
	14	++++	+++	++	+	0	0	0	120	190
Control—normal horse serum	21	0	0	0	0	0	0	++++	105	135
	22	0	0	0	0	0	0	++++	115	145
	23	0	0	0	0	0	0	++++	130	120

*Legend: ++++ flocculent precipitate, +++ slightly flocculent precipitate; ++ well-marked turbidity; + turbidity; 0 negative reaction.

TABLE 2 Desensitizing Power of Rabbit Sera Treated with Sulfanylazostarch and Sulfanylazosalicyclic Acid.

Desensitizing antigen	Guinea pig no.	Severity of anaphylactic shock	Prolongation of clotting time of the blood (in minutes)	Fall in temperature (in degrees)	Postmortem findings
Rabbit serum, treated with sulfanylazostarch	1	0	0	—0,1	—
	2	0	0(+)	0	—
	3	0	0	—0,3	—
	4	0	0	—0,1	—
	5	0	0	0	—
Rabbit serum, treated with sulfanylazosalicylic acid	6	1+	3	—1,1	—
	7	0	0	—0,1	—
	8	0	0	—0,3	—
	9	0	0	0	—
	10	0	0	0	—
Control 1—sulfanylazostarch	11	++++	—	—	+
	12	++++	—	—	+
	13	++++	—	—	+
Control 2—sulfanylazosalicylic acid	14	++++	—	—	+
	15	++++	—	—	+
	16	++++	—	—	+
Control 3—normal serum	17	++++	—	—	+
	18	++++	—	—	+
	19	++++	—	—	+

*Legend: 0) absence of reaction; + mild form of anaphylactic shock, ++++ fatal anaphylactic shock.

densensitization of guinea pigs, preliminarily sensitized with horse serum sulfanylazoglobulin. The short interval of time elapsing between the beginning of injection of the test compounds and taking the blood prevented the antibodies from being concerned in the phenomenon of desensitization. Anaphylactic shock was indiced on the 4th day after desensitization by the intracordal injection of a 4% solution of human serum sulfanylazoglobulin, in a dose of 2 ml. The results of these experiments are shown in Table 2.

Rabbit sera treated with sulfanylazostarch and sulfanylazosalicylic acid thus cause prolonged desensitization of guinea pigs, previously sensitized to horse sulfanylazoglobulin. In all controi experiments (desensitization with sulfanylazostarch, sulfanylazosalicylic acid, and normal rabbit serum), the animals died with manifestations of anaphylactic shock. These findings may be explained by the fact that the serum proteins of rabbits modify their antigenic properties under the influence of the injected azo-compounds.

It may thus be concluded from these experiments that nonprotein substances, injected into animals, denature the serum proteins, modify their immunological specificity and, in consequence of this, induce the formation of corresponding antibodies.

SUMMARY

The appearance of antibodies in response to the administration of nonprotein substances is accompanied by denaturalization of the serum proteins in the animals. The serum of rabbits injected with nonprotein substances acquires the ability to desensitize guinea pigs for prolonged periods of time. This leads to a conclusion that the introduction of nonprotein substances tends to denature the serum proteins, change their immunological specifity and, as a result of this, provoke the formation of corresponding antibodies.

LITERATURE CITED

[1] L. A. Zil'ber, Fundamentals of Immunology [in Russian] (Moscow, 1958).

[2] G. F. Marchenko and E. N. Chernyshova, Exchange of Information: Research Institutes of Vaccines and Sera of the Ministry of Health of the USSR [in Russian] (Moscow, 1955). Vol. 2, p. 27.

[3] A. I. Nikolaev, Zhur. mikrobiol., epidemiol. i immunobiol. 3, 48 (1958).

[4] F. S. Okolov, Denaturation of the Serum Proteins in the Living Animal by Immunization with Toxins, and also in Certain Diseases and Conditions in Man. Doctorate dissertation [in Russian] (Frunze, 1947).

[5] K. Landsteiner and P. Levine, J. Exper. Med. 52, 347 (1930).

[6] W. S. Tillett, O. T. Awery, and W. F. Goebel, J. Exper. Med. 50, 551, (1929).

ELECTROPHORETIC ANALYSIS OF THE COMPOSITION OF THE SPECIFIC PRECIPITATE

Yu. S. Tatarinov

Department of Biological Chemistry (Head – Prof. S. V. Zakharov) of the A. V. Lunacharskii Astrakhan Medical Institute (Director – Candidate Med. Sci. I. N. Alamdarov)

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 91-94, January, 1960

Original article submitted April 3, 1959

In numerous investigations by Heidelberger and his co-workers, the immunological specific precipitation reactions have been studied in detail [8, 9]. At the present time, some workers [4, 5] when calculating the content of antibodies in the serum, adopt the view of Kabat and Heidelberger [9], that in an equivalent albumin–antibody zone, the precipitate is composed of 90% antibody protein and 10% antigen.

V. S. Gostev and his co-workers [1, 2] who investigated the composition of the supernatant fluid after centrifugation of a specific agglutinate, found that not only had the specific proteins disappeared, but that the nonspecific proteins also were diminished. On the basis of these experiments, these authors concluded that nonspecific proteins, as well as the antigen–antibody complex, enter into the composition of the agglutinate.

The aim of the present research was, on the one hand, to determine the fractional composition of the proteins of an immune serum before and after precipitation, and on the other hand, to determine the composition of the specific precipitate by the method of microelectrophoresis on paper.

METHOD

Rabbits were immunized with solutions of pure protein fractions of normal horse serum according to a fixed program [4]. For preliminary isolation of the protein fractions, the horse serum was fractionated in a starch block (1 × 13 × 26 cm) by cold electrophoresis. The conditions of electrophoresis were: 450 v, 10 ma, duration 48 hr, veronal medinal buffer, pH = 8.6, ionic strength 0.1 μ. The purity of the fractions obtained was checked by means of paper electrophoresis.

Paper microelectrophoresis was carried out in an apparatus of the Flynn and De Mayo type, as modified by A. E. Gurvich [3]. We used B (rapidly absorptive) chromatographic paper from the Leningrad Volodarskii factory The conditions of microelectrophoresis were: potential gradient 10 v/cm, current up to 1 ma to a strip 4 × 40 cm, duration 4-8 hrm veronal medinal buffer, pH = 8.6. The electrophoregrams were stained with mercuric chloride solution of bromphenol blue of the ordinary formula; excess stain was washed out with a 2% solution of acetic acid.

The precipitating antibodies in the individual protein fractions of the immune serum were estimated by A. E. Gurvich's electrophoresis-precipitate method [4]. The precipitation reactions were performed in an expanded series, with solutions of antigen of increasing concentration, antigen solution being added to the immune serum. This order of mixing the ingredients led to a much more abundant precipitate [7]. After incubation for 30 minutes at 37°, the tubes were kept in the refrigerator for 24 hours. Electrophoretic analysis of the supernatant fluid and precipitate was performed in the equivalent zone. The precipitate was dissolved in solutions of NaOH, HCl, and urea of different concentrations, with excess of antigen.

The concentration of protein in solution and the protein content of the precipitate were estimated by Lowry's method [10]. The relative content of the protein fractions was calculated from the results of extinction (FEK-M) by the eluted dye from the electrophoregrams, cut up into fractions.

RESULTS

The table shows the summarized results obtained in 4 experiments in which the precipitation reaction was carried out with various antigens – the separate fractions of the serum proteins.

It will be seen from the figures in the table that, after precipitation, it is not only the content of γ-globulins, which include the antibodies, in the serum that was diminished, but also the other protein fractions. During precipitation with globulin antibodies, for instance different quantities of the protein fractions of the immune serum passed into the precipitate: from 46 to 51% (of the total weight of the precipitate) of γ-globulins, from 27 to 33% of albumins, and from 18 to 20% of α- and β-globulins. After careful washing of the precipitate in physiological saline and water, however, albumins could no longer be found in it; the content of α- and β-globulins was

Fractional Composition of the Specific Precipitate Obtained with Various Antigens—the Protein Fractions of Serum

Protein antigen of the blood serum			Antirabbit serum			Precipitate				
				Content of protein (in mg/ml)			relative content of protein fractions before and after washing with physiological saline			
fraction	content (in mg/ml)	volume (in ml)	volume (in ml)	before precipitation	after precipitation	quantity of protein calculated per ml of serum	albumin	globulin		
								α	β	γ
Albumins	0,50	0,15	0,15	31,0•	28,2•	2,8	41,1	13,1	13,2	32,6
						2,2••	0	8,2	10,1	81,7
Globulins										
α	0,45	0,15	0,15	34,8	30,3	4,5	33,0	9,3	9,7	48,0
						3,6	0	6,2	9,1	84,7
β	0,60	0,15	0,15	37,0	31,9	5,1	27,0	10,4	11,5	51,1
						4,0	0	8,2	9,2	82,6
γ	0,53	0,15	0,15	35,4	31,8	3,6	33,3	10,0	10,2	46,5
						3,1	0	8,7	8,6	82,7

• Each figure given is the mean of three determinations.

•• Precipitate after washing with physiological saline.

slightly decreased, and the relative content of γ-globulins was increased (to 84.7%). During precipitation of the immune serum with an albumin antigen, there was a much more pronounced fall in the albumins than in the globulins in the supernatant fluid. In the washed precipitate, no albumins could be found; the relative content of γ-globulins was also increased (to 81.7%). It should be pointed out that, even in concentrated solutions of the precipitate, the albumin antigen could not be detected electrophoretically. In this case the dissolved albumin–antibody complex had evidently acquired an electrophoretic mobility close to that of the γ-globulins (antibodies). Similar views about the dissolved antigen-antibody complexes may be found in the literature [6].

Determination of the precipitating antibodies in the various protein fractions of an immune serum by means of A. E. Gurvich's electrophoresis-precipitate method showed beyond doubt that the precipitins migrate along with the γ-globulins entirely (Fig. 1). Nevertheless, in the composition of thoroughly washed precipitate, it is possible to find not only γ-globulins, but also other protein fractions, whose electrophoretic mobility is close to that of the α_2- and β-globulins (Fig. 2). The relative contents of the latter are approximately the same (about 15%) in all the precipitates obtained with antigens of different molecular weights.

Our findings show that a comparatively large quantity of nonspecific proteins is adsorbed on the antigen-antibody complex in the process of formation of a specific precipitate with protein antigens of different molecular weights. These adsorbed proteins, however, are easily washed out with physiological saline, and the precipitate thus obtained consists mainly (about 85% of its weight) of the γ-globulins of the immune serum. A small part of the precipitate (15%) has an electrophoretic mobility similar to that of the α_2 – β-globulins. These protein fractions, firmly combined with the specific antigen-antibody complex, probably consist of nonspecific proteins.

SUMMARY

An electrophoretic analysis of the dissolved precipitate and supernatant fluid was conducted in the equivalent zone. For microelectrophoresis on paper, a Flynn and De Mayo type of chamber as modified by A. E. Gurvich was used. The specific precipitate, thoroughly washed with saline solution, largely consists of the γ-globulins of the immune serum (about 85%) and proteins, the electrophoretic mobility of which approaches that of the α_2 and β-globulins. Albumins adsorbed by the specific antigen-antibody complex are lost in washing the precipitate. A suggestion is made that about 15% of the precipitate consists of nonspecific proteins.

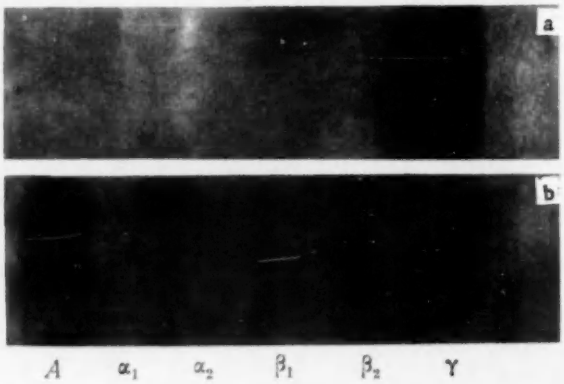


Fig. 1. Electrophoregrams of an immune rabbit serum. b) Before specific treatment by the electrophoresis-precipitate method; a) after treatment; A) albumins; α_1, α_2, β_1, β_2, γ) globulins.

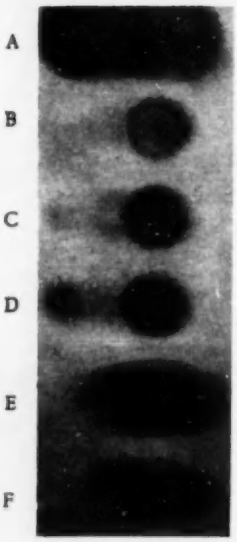


Fig. 2. Electrophoregrams of an immune serum and dissolved precipitate. A) Immune serum; B) precipitate (immediately after dissolving in N/10 alkali), washed three times; C) the same, washed twice; D) the same, washed once; E) precipitate (2 hours after dissolving), washed three times, antigen – β-globulin; F) the same, antigen – albumin from normal horse serum.

LITERATURE CITED

[1] V. S. Gostev and A. K. Saakov, Zhur. mikrobiol., epidemiol. i immunobiol. 5, 62 (1953).

[2] V. S. Gostev, O. A. Popovkina, and A. K. Saakov, Zhur. mikrobiol., epidemiol. i immunobiol. 9, 39 (1953).

[3] A. E. Gurvich, Lab. delo 3, 3 (1955).

[4] A. E. Gurvich, Biokhimiya 20, 5, 550 (1955).

[5] A. E. Gurvich and R. B. Kapner, Lab. delo 2, 23 (1958).

[6] P. Burthin, Bull. Sol. chim. 9-10, 977 (1955).

[7] J.C. Heff and E.L. Becker, J. Immunol. 3, 826 (1954).

[8] M. Heidelberger and F.E. Kendall, J. Exper. Med. 59, 519 (1934); J. Exper. Med. 62, 697 (1936); Exper. Med. 65, 647 (1937).

[9] E. Kabat and M. Heidelberger, J. Exper. Med. 66, 229 (1937).

[10] O.H. Lowry, N.J. Rosebrough and others, J. Biol. Chem. 193, 265 (1951).

INVESTIGATION OF THE ACTION OF A NUMBER OF ANTITUMOR PREPARATIONS ON CULTURES OF HUMAN TUMORS

T. G. Khaleeva

Laboratory of Experimental Chemotherapy (Head – Corresponding Member AMN SSSR Prof. L. F. Larionov) of the Institute of Experimental and Clinical Oncology (Director – Corresponding Member AMN SSSR Prof. N. N. Blokhin) of the AMN SSSR, Moscow

(Presented by Active Member AMN SSSR A. D. Timofeevskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 95-98, January, 1960

Original article submitted January 28, 1959

In the solution of problems concerned with the choice and the determination of the spectrum of antitumor preparations, transplanted animal tumors are used. Human and animal tumors, even of the same histological structure, show differing sensitivity to these preparations, however, and investigations on animal tumors do not furnish sufficient evidence on which to form conclusions on the efficacy of a drug in particular tumors in man. It is therefore necessary to seek new methods of determining, directly on human tumors, the spectrum of action of each new preparation which has been found active against animal tumors.

The use of explantation of tissues for this purpose shows great promise. The applicability of the method of cultivation of human tumors is based on the fact that, from many reports, the mechanism of the antitumor action of the majority of chemotherapeutic preparations is by their direct action on tumor cells [1, 6]. It follows that the antitumor activity of a given preparation against a given form of tumor can also be detected in vitro. Furthermore, the technique of cultivation of human tumors is now so well developed that strains of the principal human tumors are at present available for research workers, and methods have been devised for the production of single-layer cultures from biopsy material. Preparations may be tested on human cultures for periods of 30-40 hours.

The recent literature contains a number of recommendations by the leading authorities on tissue culture concerning the use of this method in the chemotherapy of tumors [4, 7, 8]. Nevertheless, in the first attempts to use the tissue culture method to detect antitumor activity in these preparations, contradictory results were obtained. Certain workers, for instance, observed differences in the sensitivity of cultures of tumor and normal tissue to the same preparation [5], whereas in other, similar investigations, no selective activity of the preparations could be found (see the survey by Hirschberg [10]). One reason for the contradictory nature of these results is, evidently, the fact that the preparations used in the investigations giving negative results possessed high toxicity, which could have reduced the sensitivity of the tumor and normal tissue to the antitumor action of the preparations.

It is also possible that other causes may be implicated in determining the results of these investigations.

Meanwhile the problem of the detection of the specific antitumor action of preparations in tissue culture conditions remains unsolved. The aim of the present investigation was to study this problem.

METHODS

A special feature of our experiments is that, besides studying preparations already known, we also tested new ones, created in accordance with the principle of complex alkylating metabolites, enunciated by L. F. Larionov [2], namely asalin, phenaphan, and phenameth. In animal experiments these drugs showed high antitumor activity, a varying degree of specificity of their action on tumors [3] and, at the same time, comparatively low toxicity.

The chemical names of these preparations are as follows: asalin is N-acetylsarcolysinvaline (ethyl ester); phenaphan is p-di(2-chloroethyl)aminophenacetylphenylalanine (ethyl ester); phenameth is p-di(2-chloroethyl)aminophenacetylmethionine (ethyl ester).

We also investigated the action on tissue cultures of acetylsarcolysin and chlorophenacyl [p-di(2-chloroethyl)aminophenylacetic acid], which enters into the composition of these three preparations, and also of three previously known preparations – embichin, DL--sarcolysin, D-sarcolysin, and thio-TEPA.

We had available cultures of a strain of carcinoma of the uterine cervix (HeLa), obtained from Gey [9], cultures of normal epithelium from a human embryo (from A. D. Timofeevskii's laboratory 1958), and also cultures of the normal endothelium of the heart of a Cinomolgus monkey, obtained in the USSR in D. E. Solk's laboratory in 1957. First of all we attempted to discover any differences in the sensitivity of tumor and normal

tissue to equal doses of the same preparation under explantation conditions, i.e., if there was any selectivity in the action of the preparation on the cultures of tumor cells.

The strains used were cultivated by the usual method on synthetic medium 199, with the addition of human serum (20%) for carcinoma of the cervix uteri and for normal human epithelium, and also ox serum (20%) for the endothelium. Cultivation was carried out in Carrel flasks, 4-5 cm in diameter. After growth for 5 days, the prospective preparations were added in different doses to the fresh nutrient medium of the experimental cultures. The period of action was 24-48 hours, after which the cultures were photographed; next, a proportion of the flasks was treated with trypsin solution (DIFCO), the detached cells were centrifuged at 1000 rpm for 3 minutes, fresh nutrient medium was added to the precipitate, and the experimental cultures were again allowed to grow. The results of the action of the preparations were assessed by the ability of the culture to grow in the second generation. The maximum dose at which the cells grew in the form of colonies was defined as the "maximum dose of positive passage". If the cells were found to be incapable of further growth in new flasks, the minimum dose of the preparation causing these changes was designated the "minimum lethal dose". Naturally, the more important value in defining the antitumor activity of the preparations was the maximum dose of positive passage, and this value was compared in different series of experiments.

RESULTS

The results obtained from our experiments are shown in Table 1. It can be seen that the cultures of normal epithelium and endothelium possessed different sensitivity to the test preparations. From a comparison of the action of the same preparations on carcinoma of the uterine cervix and on epithelium, for instance, the highest selective antitumor action was shown by phenaphan and acetylsarcolysin; in response to the action of these preparations, the tumor culture retained its power of growth in the second generation only with doses of 125 and 100 γ/ml, whereas the culture of epithelium was capable of passage after the action of doses as high as 500 γ/ml of phenaphan and 400 γ/ml of acetylsarcolysin. Comparative evaluation of the action of these two preparations on carcinoma of the uterine cervix and on endothelium showed that asalin had the highest selectivity of action; after the action of this preparation a second generation of tumor cells grew only with a dose of 50 γ/ml, but cultures of endothelium kept the power of passage even with a dose of 200 γ/ml.

It follows from Table 1 that 6 of the 9 preparations studied, namely all the alkylating metabolites except DL-sarcolysin, had a stronger action on the culture of tumor cells than on the cultures of normal cells, for the maximum dose of positive passage was half as great for the tumor cells as for normal cells.

TABLE 1 Comparative Action of Chemical Preparations of the Chloroethylamine Group on Cultures of Malignant and Normal Cells

Preparation	Maximum dose of positive passage		
	carcinoma of the cervix (HeLa)	human epithelium	endothelium of the monkey's heart
Embichin	25	12.5	12.5
DL-Sarcolysin	25	25	25
D-Sarcolysin	100	200	200
Chlorphenacyl	50	50	200
Phenaphan	125	500	250
Phenameth	100	200	200
Asalin	50	100	200
Acetylsarcolysin	100	400	200
Thio-TEPA	50	100	50

TABLE 2 Comparative Action of Chemical Preparations of the Chloroethylamine Group on Different Strains of Human Malignant Tumors in Tissue Culture

Preparation	Maximum dose of positive passage (in γ/ml)		
	carcinoma of the uterine cervix (HeLa)	carcinoma of the larynx (HEr = 2)	angiosarcoma (AS)
Embichin	25	12.5	12.5
DL-Sarcolysin	25	12.5	25
D-Sarcolysin	100	50	50
Phenacyl	50	50	50
Phenaphan	125	125	1000
Phenameth	100	100	400
Asalin	50	50	200
Thio-TEPA	50	25	50

It is also apparent from Table 1 that DL-sarcolysin had no selective action on the tumor cultures, and that embichin had a stronger action on the epithelium and endothelium than on the tumor cells. So far as chlorophenacyl and Thio-TEPA are concerned, the selectivity of action of Thio-TEPA was shown by comparison of its action on tumor cultures and cultures of normal epithelium, and the antitumor action of chlorophenacyl was shown by comparison of its action on the same tumor cells and the culture of endothelium.

The new antitumor preparations which we studied, belonging to the group of "complex alkylating metabolites", thus possess greater selectivity of action than the antitumor preparations previously known.

The difference in the sensitivity of the tumor and normal tissues to these antitumor preparations during explantation enabled us to determine the spectrum of action of these preparations on human tumors in these

conditions. We had available 3 strains of human malignant tumors – carcinoma of the uterine cervix (HeLa), carcinoma of the larynx (HEr = 2) and angiosarcoma (AS), and these were used in the investigation. The results obtained are shown in Table 2.

From a comparison of the maximum doses of positive passage it can be seen that 2 of these strains – – carcinoma of the uterine cervix and carcinoma of the larynx – were more sensitive to 3 of the 8 preparations tested, namely to the "complex alkylating compounds" phanaphan, asalin and phenameth, whereas angiosarcoma continued to grow in the second generation even after the action of a large dose. It may be postulated from these findings that these particular preparations showed greater specificity of action on certain variants of squamous-cell carcinoma than on sarcoma. With regard to the preparations used, the method of tissue culture of human tumors thus gave a provisional answer to the question of which human tumors might be expected to show therapeutic effects from the use of these preparations.

Our experiments also revealed a difference in the action of the preparations on the different epithelial tumors. Carcinoma of the larynx, for instance, was more sensitive to D-sarcolysin and thio-TEPA than was carcinoma of the uterine cervix.

It follows from these results that we succeeded in demonstrating an appreciable difference in the maximum doses of positive passage of certain of the antitumor preparations when used on tissue cultures of human tumors. The suggestion, made above, that it may be possible to use the tissue culture method to detect not only antitumor activity but also the spectrum of action of the preparations examined, was thus confirmed. Comparison of our results on cultures in vitro with the results of clinical trials of these particular preparations will indicate to what extent this method may be used for practical purposes.

SUMMARY

Experiments were conducted on cultures of human tumor cells – of cancer of the uterine cervix (HeLa), cancer of the larynx HEr-2, and angiosarcoma (AS), as well as on those of cardiac endothelium of the "Cinomolgus" monkey. The author studied the action of the new Soviet antitumor preparations asalin, phenaphan, phenameth, acetylsarcolysin, and phenacyl, as well as of the existing preparations – embichin, dl-sarcolysin, d-sarcolysin and thio-TEPA. Investigations revealed that differences in the sensitivity of the tumor and normal tissue to the same preparations are also present in tissue culture. Thus, the effect of the complex alkylating metabolites (excepting dl-sarcolysin) on tumour cell cultures was 2–4 times greater than that exerted on cultures of normal cells. The varying effect of the preparations under study on different tumors could be also ascertained in tissue cultures. Thus, carcinoma of the uterine cervix and of the larynx appeared to be more sensitive to asalin, phenaphan, and phenamethane than did angiosarcoma, while the carcinoma, of the uterine cervix proved to be more resistant than carcinoma of the larynx to d-sarcolysin and Thio-TEPA.

LITERATURE CITED

[1] L. F. Larionov and M. A. Presnov, Arkh. patol. 20, 1, 32 (1958).

[2] L. F. Larionov, Vestnik akad. med. nauk SSSR 2, 8 (1958).

[3] Z. P. Sof'ina, Proceedings of the second All-Union Oncological Conference [in Russian] (Leningrad, 1958) p. 216.

[4] A. D. Timofeevskii, Patol. fiziol. i Eksptl. terapiya 1, 3, 6-14 (1957).

[5] A. D. Timofeevskii, Etiology and Pathogenesis of Tumors [in Russian] (Moscow, 1957) p. 79.

[6] L. F. Sharlikova, The Development of Drug Fastness in Sarcoma 45 of Rats to Chloroethylamines and Ethylamines. Candidate's dissertation [in Russian] (Moscow, 1958).

[7] A. M. Brues, J. National Cancer Inst. 19, 771 (1957).

[8] J. Biesele, Scient. Am. 195, 50 (1956).

[9] G. O. Gey, W. D. Coffman, and M. T. Kubicek, Proc. Am. Ass. Cancer Res. 12, 264 (1952).

[10] E. Hirschberg, Cancer Res. 18, 869 (1958).

REGENERATIVE HYPERTROPHY OF THE SKIN IN RATS

Yu. K. Man'ko

Laboratory of Growth and Development (Head – Prof. L. D. Liozner) of the Institute of Experimental Biology (Director – Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 99-103, January, 1960

Original article submitted April 14, 1959

In recent times the problem of regeneration of the mammalian skin has undergone considerable reappraisal [1-3]. It has been increasingly found that the processes taking place after the infliction of a wound cannot be dismissed simply as the drawing together of the edges of the wound and the formation of a scar. A subject that is particularly worthy of attention is that of the restorative processes taking place in the immediate surroundings of the wound.

The power of growth, shown by areas of skin of the adult animal after the infliction of a wound, was clearly demonstrated by Billingham and Medawar [5]. These workers found a considerable increase in the dimensions of small areas of skin which they left, in the form of islands, in the center of skin wounds in rabbits. The original thickness of the skin was maintained in the course of this process.

Experiments of a similar character were performed by I. V. Markelova [4] on albino rabbit. She also left islands of skin in the center of a square wound. One month after operation, the area of the islands had increased on the average threefold by comparison with their original area. I. V. Markelova further showed that reparative phenomena are observed not only in the skin of the island but also in the skin surrounding the wound.

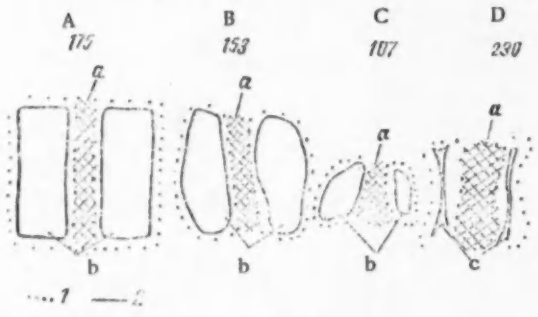


Fig. 1. Scheme of the operation in the first series of experiments and the sequence of changes in the area of the piece of skin (a) situated between the two wounds. A) Scheme of the operation; B,C,D) disposition of the wounds and ink marks in rat No. 8 at different times after infliction of the wound: B) after 24 hours; C) after 10 days; D) after 60 days. a) Measured area of skin (shaded); b) wounds; c) scars. The figures above the figures indicate the area of the piece of skin (a) in square millimeters.

In view of the inadequate study of this problem, it was important to ascertain whether these manifestations of interposed growth, observed after the infliction of a wound, are peculiar to rabbits or are also to be found in other mammals; whether, in particular, they occur in rats, in which animals it is generally accepted that the process of wound healing takes place exclusively by cicatrization and drawing together of the wound edges.

METHODS

Experiments were carried out on male white rats weighting 170 g. In order to ascertain the changes taking place in the skin adjoining the wound in the process of healing, two series of experiments were performed. In the first series we studied the changes in the piece of skin situated, in the form of strip, between two wounds inflicted on the animal's back; in the second series of experiments observations were made on the zones of skin surrounding the wound. We first tried to leave a small island of skin in the center of the wound, but we had to abandon this method for the island was always found to die. We accordingly had to resort to the modification of the experiment described above.

In the first series of experiments we used 8 animals. A tattoo mark, in the form of a square, was made on the rat's back, through a stencil, and in the center of the mark, two further rows of marks were inscribed (in ink, with the point of a needle). In the spaces between the marks, two wounds were cut, so that a strip of skin was left between them in the form of an isthmus (Fig. 1, A). The skin was excised together with the cutaneous muscle. The size of the square was 3.5 x 3.5 cm, the width of the strip of skin 0.5 cm and its length 3.5 cm. It was necessary to make a preliminary

tattoo mark because, during the healing of the wounds, their outlines of their edges and the shape of the strip underwent considerable changes. The method by which we carried out the experiments enabled us to observe the changes in the area of a definite piece of skin, delineated by ink marks (see Fig. 1, A, B, C, D-a).

The outlines of the wound and the disposition of the marks were systematically traced at various times after operation on to a celloidin film, and the tracing was then transferred to squared paper.

RESULTS

The wounds healed under a dry scab. Their dimensions decreased progressively as a result of indrawing of their edges. After removal of the scab (25-28 days after operation), scars were revealed, covered with young epidermis, considerably smaller in area than the original wounds. In the course of the first 10-20 days, the area of the strip of skin between the wounds decreased on the average by 30%, evidently on account of poor conditions of nutrition. After healing of the wounds, however, the strip began to grow. Thirty-seven days after operation the area of the strip was on the average, 124% its original area, and 4 months after operation it was 172%. In some rats the growth of the strip was particularly well marked; in one, for example, the area of the strip 4 months after the operation was 275% of the original area. This increase in the dimensions of the strip of skin left between the wounds cannot be explained purely by growth of the rats. In this space of time, the distance between marks on the back of rats of the same size, not undergoing operation, increased by only 20%.

The results of the first series of experiments thus revealed a more intensive growth of the skin situated between the two wounds; this, in our opinion, should be regarded as a restorative reaction, developing in response to the removal of an area of skin.

In order to ascertain whether the increase in the area of the skin situated between the wounds was the result of mechanical stretching, we made a histological examination of areas of skin taken at the beginning and end of the experiment. In the first case we used the piece of skin excised when the wound was made, and in the second case, pieces taken from the strip of skin itself. In the histological preparations attention was directed towards the general structure of the skin, its thickness was measured, and the number of hair groups and the number of hairs in each group were counted. The thickness of the skin and its structure in rodents are known to change considerably, depending on the phase of the growth cycle of the hair. This factor was also taken into consideration when the preparations were examined.

The results obtained showed that the skin structure was practically unchanged during the time of the experiment; its thickness also remained unchanged (Table 1).

It can be seen from Table 1 that no change was caused in the thickness of the skin by the infliction of a wound and the processes connected therewith. In those cases in which the skin was in the same stage of the growth cycle at the beginning and the end of the experiment (rat No. 8), its thickness was the same.

TABLE 1 Changes in the Thickness of the Skin of Rats during the Experiment

Rat No.	Time	Stage of hair growth	Thickness of skin (in μ)
1	Beginning of experiment (15/VII)	Rest	7.5
	End of experiment (21/XI)	Intensive growth	10
3	Beginning of experiment (15/VII)	Rest	12
	End of experiment (21/XI)	Intensive growth	10
6	Beginning of experiment (15/VII)	Rest	12.5
	End of experiment (21/XI)	Intensive growth	10
8	Beginning of experiment (15/VII)	Rest	12.5
	End of experiment (21/XI)	Intensive growth	12.5

The number of hair groups found in one field of vision (counted in tangential sections) fell by 24% at the end of the experiment. This fall, however, was not of the same order as the increase in the area of the strip of skin.

The number of hairs in the hair groups was practically unchanged. In the original piece of skin, from 5.2 to 6.9 were counted, and in the piece of skin taken at the end of the experiment there were 5.4 to 6.6. We were unable to detect any difference in the structure of the epithelium, or in the thickness and distribution of the collagen fibers in the pieces of skin fixed at the beginning and end of the experiment. The more intensive growth of the piece of skin situated between the two wounds evidently took place as the result of processes similar to the growth processes in the undamaged skin of the animal.

The second series of experiments was carried out on 4 rats. A wound, measuring 2.5 × 2.5 cm, without an island, was produced in the middle of the animal's back. Ink marks were applied in a direction from the wound edge outwards, at intervals of 7-8 mm (Fig. 2, A). The marks were traced throughout the experiment.

The wounds decreased in size asymmetrically during healing (the transverse measurement shortened the most). The scar formed after healing of the wound was always elongated in shape. During and after the healing period, the distances between neighboring marks were observed to increase gradually; in other words, on account of growth of the skin, the marks became further

TABLE 2 Changes in the Distances Between the Marks in the Course of the Experiment, in Percentages of the Initial Distance

Zone	Before wounding	Time (in days)					
		5	10	15	26	77	182
Ia	100	111	127	139	148	174	181
Ib	100	109	116	123	126	142	139
II	100	108	109	113	128	123	127
III	100	105	102	107	119	127	116

apart from each other. This process took place irregularly. Areas of skin situated nearest the wound, especially at its sides, underwent the most intensive growth. This was in accordance with the decrease in the size of the wound mainly in the transverse direction (Fig. 2, B).

In Table 2 we give the figures showing the changes in the distances between the marks (mean values for 4 animals). The figures are given collectively for the individual zones, for in this way they reveal the characteristic trend more clearly. We designated zone I the distance between the edge of the wound and the 2nd row of marks, zone Ia including the marks situated at the sides of the wound, and zone Ib the marks anterior and posterior to the wound; zone II, the distance between the 2nd and 4th rows of marks, and zone III the distance between the 4th and 6th rows of marks (see Fig. 2).

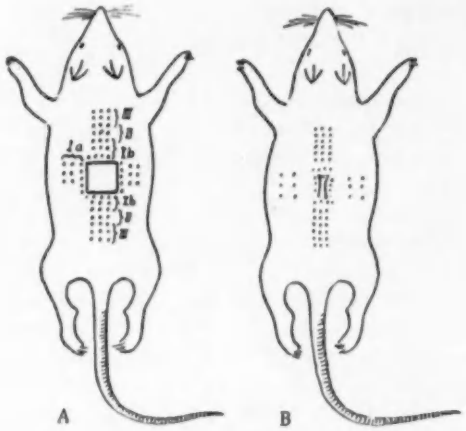


Fig. 2. Results of the second series of experiments. A) Initial disposition of the marks on the rat's back; B) disposition of the marks at end of the experiment. The Roman numerals designate the zones including the measured areas of skin.

Corresponding to the fact that the transverse measurements of the wound fell sharply, the distance between the marks of zone Ia showed a large increase; the distance between the marks in zone Ib, although it increased more than that in zones II and III, did not increase to the same extent as that in zone Ia.

In order to ascertain whether the unequal growth of the areas of skin in the rats undergoing operation was not the result of the growth of the animals themselves, we carried out the following experiment. A square was tattooed in the center of the back of 3 control rats in just the same way as in the experimental rats, and parallel rows of marks were applied in an outward direction from the square on the 4 sides; no wounds, however, were excised.

It must be mentioned that this experiment was conducted at a different time of year (November-January) and on younger animals. Measurements taken after 40 and 110 days gave the following results (Table 3).

In rats not undergoing operation, the growth of the skin in the different zones thus proceeded more or less uniformly. From this it can be concluded that the uneven growth of the areas of skin in the rats with wounds of the dorsal region was due to growth of the skin surrounding the wound, under the influence of wounding.

On the whole, the results show that in rats, just as in rabbits, the healing of extensive skin wounds is accompanied not only by mechanical drawing together of the wound edges, but also by a more intensive growth of the skin in the vicinity of the wound, leading eventually to the filling up of the defect. This reparative process may be described as regeneration, taking place by means of regenerative hypertrophy. By this term is understood the restoration of the volume and the func-

TABLE 3 Changes in the Distances Between the Marks on the Back of Rats Not Undergoing Operation (in percentages of the initial distance)

Zone	Time (in days)	
	40	110
Ia	120	132
Ib	105	135
II	118	139
III	128	128

tion of an organ, by means of processes of growth and development, taking place in the remnant of the organ, at the same time as a connective tissue scar is formed on the surface of the wound. The healing of skin wounds cannot thus be regarded simply as the formation of a scar, for, besides the drawing together of the wound edges, leading to closure of the wound, an increase is observed in the mass of the skin surrounding the wound, as a result of which the thickness of the skin remains as before.

SUMMARY

The measurement of the area of the skin located between two wounds on the back of rats and delimited by India ink marks, has demonstrated that with time it increased considerably, in 4 months time constituting 175% of the initial area. Observation of India ink marks made at a definite distance around the wounds demonstrated that the infliction of the wound intensified the growth of the areas of the skin adjacent to the wound. The initial thickness of the growing skin was preserved, and the number of the hair follicle groups was somewhat reduced. The data obtained demonstrated the development of restorative processes of the regenerative hypertrophy type in the skin surrounding the wound.

LITERATURE CITED

[1] M. E. Aspiz, Restoration of Tissues and Organs [in Russian] (1954) pp. 92-113.

[2] A. A. Braun, Izvest. akad. nauk SSSR, ser. biol. 6, 695-698 (1945).

[3] M. A. Vorontsova and L. D. Liozner, Asexual Reproduction and Regeneration [in Russian] (Moscow, 1957).

[4] I. V. Markelova and Yu. K. Tonkonogova, Problems of Regeneration and Cellular Proliferation [in Russian] (Moscow, 1959).

[5] R. E. Billingham and P. B. Medawar, J. Anat. 1, 114-123 (1955).

THE INFLUENCE OF INFLAMMATION ON THE REGENERATION OF MUSCLE IN EXPERIMENTS WITH MINCED MUSCLE TISSUE

R. P. Zhenevskaya

Laboratory of Histology (Head – Prof. A. N. Studitskii) of the A. N. Severtsov Institute of Morphology of Animals (Director – Corresponding Member AN SSSR Prof. G. K. Khrushchev) of the AN SSSR, Moscow

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 104-110, January, 1960

Original article submitted June 29, 1959

The method of restoration of muscles by means of autotransplantation of minced muscle tissue, suggested by A. N. Studitskii [12, 13] and developed by a group of his co-workers [3-6, 9-11], has attracted increasingly the attention of research workers and has begun to be applied in several laboratories. In a series of papers [1, 2, 8, 15], the positive results of transplantation of minced muscle tissue in mammals and even in man are described. Meanwhile, in the investigations of Klika and Doskocil [16], homotransplantation of ground embryonic muscle tissue to the site of damage to a muscle had no very obvious effect; this led these authors to have doubts regarding the use of transplanted material in the regeneration of muscle tissue.

G. M. Litver and N. N. Dampel' [7] have expressed themselves even more categorically, asserting that minced muscle tissue, grafted at the site of an excised muscle, takes no part in its regeneration, but simply provokes an inflammatory reaction, as a result of which it undergoes absorption. These conclusions, in our view, are due to failure to observe the details of the operative technique which we have developed. In a special article, devoted to the restoration of muscle by means of grafting with minced muscle tissue [14], the method of the operation was described in detail and attention was drawn to the necessary conditions for successful regeneration of muscle. One of these essential conditions is the sterility of the operation, preventing suppuration, which sharply inhibits the regeneration of muscle. A no less essential condition for the successful regeneration of muscle by means of this method is the proper mincing of the muscle tissue. Drying of the material, overheating it, or other factors leading to necrosis of the grafted tissue will also cause a more intensive inflammatory reaction.

The present research was undertaken in order to show the importance of inflammation as a condition preventing the process of regeneration in experiments with minced muscle tissue.

METHODS

Two series of experiments were performed on white rats aged 2½-3 months. In the first series, the gastrocnemius, soleus, and plantaris muscles of the animals were excised by division at the level of the tendons. The neurovascular bundle was preserved. Minced muscle tissue, taken from the lateral head of the gastrocnemius muscle, was placed in the bed of the excised muscles. The whole operation was performed in strictly sterile conditions. In the second series of experiments, the animals underwent the same operation, but without aseptic precautions. In order to intensify the inflammatory process and to stimulate suppuration, balls of cotton wool, 0.5-0.8 mm in diameter, were mixed with the minced muscle tissue. Fixation was in Zenker's fluid and in 12% neutral formalin, 10, 21, 30, and 60 days after operation. Sections were stained with azocarmine by Heidenhain's method, with Regaud's iron-hematoxylin, and with hematoxylin and counterstaining by Mallory's method. Part of the material was treated with silver by the Bielschowsky-Gros-Lavrent'ev method.

RESULTS

A comparative analysis of the results obtained in the two series of experiments clearly demonstrates the role of inflammation in the regeneration of muscle from minced muscle tissue. In many animals, in the second series of experiments, on naked-eye examination of the regenerating muscles suppuration was found. The results given in the table show the change in weight of the regenerating muscles in the process of their development. In the first stage of regeneration – 10 days after autotransplantation – the weight of the regenerating muscles showed little difference in the two series of experiments; the mean weight of the regenerate in the presence of experimental inflammation was 324 mg, and in sterile conditions 333 mg. By the end of the 3rd week, however, the weight of the regenerates in the series with experi-

Change in Weight of the Regenerating Muscles in Experiments with Minced Muscle Tissue

Serial no.	In sterile conditions		Accompanied by experimental inflammation	
	Time after operation, in days	Weight of regenerate, in mg	Time after operation, in days	Weight of regenerate, in mg
1	10	320	10	220
2	10	300	10	420
3	10	300	10	180
4	10	450	10	350
5	10	340	10	450
6	10	300	—	—
7	10	320	—	—
		Mean 333		Mean 324
1	21	170	21	150
2	21	100	21	60
3	21	190	21	180
4	21	150	21	90
5	21	150	21	70
		Mean 152		Mean 110
1	30	250	30	60
2	30	200	30	120
3	30	170	30	190
4	30	180	30	130
5	30	270	—	—
		Mean 214		Mean 125
1	60	310	60	170
2	60	270	60	150
3	60	300	60	120
4	60	200	60	60
5	60	290	60	190
6	60	310	60	160
7	60	300	60	170
8	60	380	60	120
9	60	310	60	170
10	60	180	60	150
11	60	400	60	110
12	60	300	—	—
13	60	460	—	—
14	60	350	—	—
15	60	370	—	—
		Mean 315		Mean 142

mental inflammation began to fall appreciably behind that in the control series (110 mg in the series with inflammation and 152 mg in the control series); 2 months after the operation, the regenerates of the control series weighed more than twice those obtained in the series with inflammation (315 mg in the control series and 142 mg in the series with experimental inflammation).

The outward appearance of the regenerates differed not only in their size but also in their shape and color (Fig. 1). Regenerates after 2 months under sterile conditions had the appearance of a muscular organ, dark pink or red in color (dark on a light background); in the series with stimulation of suppurative inflammation, thin bands of a white or yellowish hue were obtained (light on a dark background). Microscopic examination showed differences in the course of the regenerative process in the two series of experiments.

Ten days after operation, in the first series, carried out in sterile conditions, the regenerating muscles were in the stage of transition from the myo-

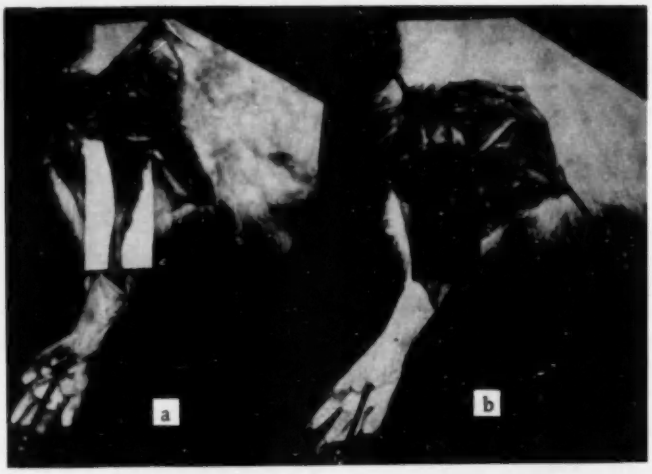


Fig. 1. Regenerates 2 months after autotransplantation of minced muscle tissue. a) In sterile conditions; b) accompanied by experimental inflammation.

blastic phase of development into the myosymplastic phase. The transplanted material was now undergoing transformation, the inflammatory reaction was feebly developed and a change to a proliferative process was apparent. The regenerating muscle was found to contain streams of myoblasts and bundles of muscle tubules, some having longitudinal fibrils, and crossing the regenerate in various directions. Those parts of the grafted muscle tissue which had not undergone progressive change were disappearing, and were almost completely absorbed, isolated fragments being seen here and there. Numerous small blood vessels penetrated into the thickness of the regenerating muscle. The commoner cells to be found in the blood and connective tissue filling the spaces in the areas where the myoblasts and muscle tubules were less compact, were lymphocytes, monocytes, and polyblasts of histogenous and hematogenous origin, with nuclei of a round or bean-shaped appearance. Fibroblasts and very fine, delicate collagen fibers could be seen.

In the second series, accompanied by experimental inflammation, the regenerating muscles contained extensive areas of unabsorbed, highly necrotic fragments of transplanted muscle tissue, mixed with cotton wool fibers. These areas were infiltrated with an enormous number of leukocytes, which surrounded them with a continuous leukocytic barrier. Here were to be found neutrophils with segmented and annulated nuclei, lymphocytes, monocytes, and numerous polyblasts, of various shapes and sizes, with compact, round nuclei. There were a few blood vessels in the regenerating muscle. In places, a young connective tissue had developed, containing fibroblasts and thin collagen fibers. Sometimes a thin layer of connective tissue surrounded areas with remains of necrotic portions of muscle fibers. A repair reaction in the muscle tissue was apparent only at its proximal end, in the remnant of the excised muscle; here were found a small number of myoblasts and muscle tubules. The latter turned back upon themselves on meeting fragments of necrotic muscle tissue, surrounded with a connective tissue capsule, in their path.

On the 21st day, the regenerates obtained in the first series of experiments contained thin, cross-straited muscle fibers and muscle tubules, forming a plexus; muscle cells sometimes lay in compact bundles, and in other places were arranged less closely together. In these areas could be seen blood vessels, close to which were found isolated, small fat cells. The muscle fibers and tubules were interspersed with fine collagen fibers, which were entwined between them. Only very rarely could isolated fragments of grafted muscle fibers be found in the regenerates, which had not undergone progressive development.

In the second series, the regenerates appeared to consist mainly of connective tissue; in places this tissue was loose and contained many cells, mainly fibroblasts. In other areas were dense bundles of collagen fibers. Here and there the regenerate was penetrated by blood vessels. Islands of necrotic fragments of transplanted muscle tissue were found in the regenerates, infiltrated by many leukocytes of various forms, polyblasts, and macrophages. Here also were occasional fibroblasts and very fine collagen fibers. A noteworthy feature was the appearance, close to the blood vessels, of numerous groups of large fat cells, round or polygonal in shape, with small, compact, unsegmented nuclei containing coarse granules, staining a yellow color with the azan method. The regenerates contained a very small number of newly formed muscle cells – myoblasts, muscle tubules, and young, slender, cross-striated muscle fibers with large nuclei.

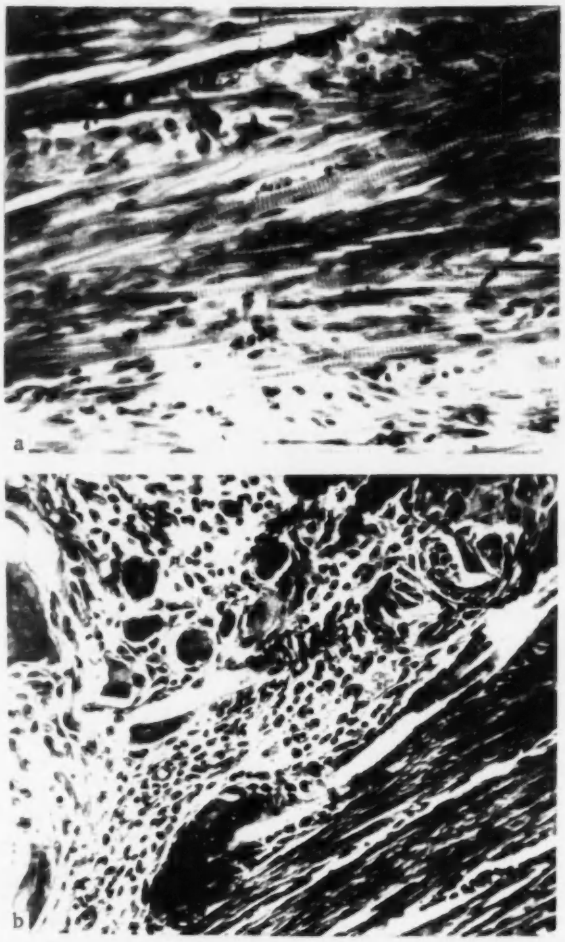


Fig. 2. Structure of the regenerating muscles 21 days after autotransplantation of minced muscle tissue into the bed of the excised muscles. Microphotograph. a) In sterile conditions (cross-striated muscle fibers differentiated; b) accompanied by experiment inflammation (area containing necrotic fragments of muscle fibers, infiltrated with leukocytes, polyblasts, and macrophages; below, on the right—fibrous connective tissue).

Thirty days after operation, the regenerates obtained under sterile conditions (first series) consisted almost entirely of interwoven and completely differentiated muscle fibers, larger in diameter than at the time of the previous examination. Signs that the repair reaction was not yet complete were found in the presence of numerous chains of muscle nuclei. The bundles of muscle fibers were separated by small connective tissue septa.

In the presence of experimental inflammation (second series of experiments) the regenerates consisted of connective tissue — either fibrous, forming compact bundles of relatively thick collagen fibers, or loose, containing fibroblasts, fat cells, and collections of mast cells. Only occasional cross-striated muscle fibers were present in the regenerate, mainly at its proximal end. In the center of the regenerates could clearly be seen necrotic fragments of transplanted muscle fibers, surrounded by cells of various types from the purulent exudate.

We were especially interested in the examination of the regenerated muscles 60 days after operation, as representing the final result of the process of repair. At this time, we examined the largest number of animals (see Table). The regenerates were stained by the silver method, to show nerve endings, as well as by the usual histological methods.

In the first series the regenerates consisted of interwoven, differentiated muscle fibers; these were still larger in diameter, showed obvious cross-striation and often contained long chains of nuclei. At the proximal end and in the center of the regenerate were seen a few fine septa of collagen fibers, which merged at the distal end with the tendo Achillis. Here and there were found small areas of loose connective tissue containing blood vessels and fat cells; very occasionally solitary, small fat cells were seen, which had lost their granularity. The regenerated muscle was richly supplied with nerve cells. A well-developed plexus of nerve trunks and single nerve fibers penetrated into the thickness of the regenerate, most of which had a formed myelin sheath. The nerve fibers terminated in motor end-plates in varying stages of maturity. They showed great variation in the form, the number, and the degree of branching of their endings and in the number of nuclei of the end-plate. The terminal branches ended in fairly large loops, such as are characteristic of regenerating motor end-plates.

In the second series, the regenerates consisted of bands of fibrous connective tissue, between the bundles of which, occasional cross-striated muscle fibers could here and there be found. In the areas of looser connective tissue there were blood vessels, fibroblasts, cells with annulated nuclei, and a still considerable number of fat cells. In some regenerates at this time, remains of necrotic fragments of the transplanted tissue could be seen, mingled with traces of cotton wool fibers. These areas were filled with infiltrations of small cells, among which could be seen many degenerating cells. In the region of the newly formed muscle fibers were seen regenerating nerve fibers, some of which had a myelin sheath, although the majority were bare axis-cylinders. These had simple endings in the form of "buttons," glomeruli, spirals or primitive motor end-plates. More mature motor end-plates were only very rarely observed.

From the foregoing it can be concluded that the character of the inflammatory process is of great importance for the regeneration of muscle from minced muscle tissue.

In the series of experiments carried out in strictly sterile conditions, the greater part of the transplanted

material underwent radical reorganization, resulting in the development of muscle cells and the formation of a typical muscle organ; this was differentiated into striped muscle fibers, interspersed with a small amount of connective tissue which merged into the tendon at the distal end of the muscle. The newly formed muscle was richly supplied with nerves and equipped with mature motor end-plates.

In the presence of suppurative inflammation, the processes of progressive development of the particles of transplanted material and formation of muscle and nerve cells were inhibited, the absorption of necrotic fragments of the minced muscle fibers was long delayed, and the productive development of connective tissues was stimulated. The regenerate formed as a result of the inflammatory process was no more than a connective tissue reproduction, incorporating only a small number of striped muscle fibers, supplied with very primitive motor nerve endings.

It must therefore be recognized that, besides tension and intact neuromuscular connections, an essential condition for the successful and perfect restoration of whole muscles by the method of autotransplantation of minced muscle tissue is the performance of the operation in conditions excluding the development of a suppurative inflammatory process.

SUMMARY

Experiments (2 series) were conducted on white rats. The significance of the characterof inflammatory process for the restoration of the muscles was demonstrated by the method of autotransplantation of minced muscular tissue. Only isolated fragments of material transplanted in place of removed muscles of the posterior extremity underwent narcosis and resolutions the greater part of the minced muscular tissue appeared to be viable and underwent radical reconstruction, bringing about the development of myoblasts, muscle tubes, and differentiated cross-striated muscle fibers supplied with nerve elements. A typical muscular organ was formed as a result of restorative process. Necrosis of minced muscular fibers was intensified in the presence of purulent inflammation; their progressive development and the resolution of necrotic particles became sharply retarded and the productive development and the resolution of necrotic particles became sharply retarded and the productive development of connective tissue, resulting in the formation of connective tissue reproduction at the site of the transplant, was stimulated

LITERATURE CITED

[1] K. I. Gavrilova, L. S. Romanova, and A. S. Gurvich, Eksptl. khirurgiga 4, 14 (1958).

[2] G. L. Emts, Trudy Ukrain. tsentr. nauchno-issled. inst. ortopedii i travmatologii 6, 41 (1955).

[3] R. P. Zhenevskaya, Doklady akad. nauk SSSR 96, 1, 217 (1954).

[4] R. P. Zhenevskaya, Izvest. akad. nauk SSSR ser. biol. 6, 90 (1955).

[5] R. P. Zhenevskaya, Doklady akad. nauk SSSR 121, 1, 182 (1958).•

[6] Z. P. Ignat'eva, Doklady akad. nauk SSSR 100, 5, 1017 (1955).

[7] G. M. Litver and N. N. Dampel', Proceedings of the Sixth All-Union Congress of Anatomists, Histologists and Embryologists [in Russian] (Kiev, 1958) p. 334.

[8] E. E. Malovichko and S. I. Voronchikhin, Collected Author's Abstracts and Theses of Scientific Work of the Izhevsk State Medical Institute [in Russian] (1956) p. 218.

[9] O. N. Rumyantseva, Doklady akad. nauk SSSR 96, 4, 869 (1954).

[10] O. N. Rumyantseva, Arkh. anat. gistol. i embriol. 33, 3, 26 (1956).

[11] R. V. Samsonenko, Arkh. anat., gistol. i embriol. 33, 2, 60 (1956).

[12] A. N. Studitskii, Doklady akad. nauk SSSR 84, 2, 389 (1952).

[13] A. N. Studitskii, Trudy inst. morfol. zhivotnykh im. A. N. Severtsova Akad. Nauk SSSR 11, 225 (1954).

[14] A. N. Studitskii, R. P. Zhenevskaya and O. N. Rumyantseva, Ceskoslovenska Morfologie 4, 4, 331 (1956).

[15] S. Hoja, Biologia 13, 7, 514 (1958).

[16] E. Klika and M. Doskocil, Ceskoslovenska Morfologie 4, 1, 10 (1956). p. 10.

THE EFFECT OF IRRADIATION OF THE RECIPIENT WITH X-RAYS ON HOMOPLASTIC TRANSPLANTATION OF A PERIPHERAL NERVE

Yu. M. Gudzovskaya

Department of Histology (Head – Prof. A. A. Braun) of the Kirghiz Medical Institute, Frunze

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 110-114, January, 1960

Original article submitted June 29, 1959

The peripheral nerves possess well-known powers of regeneration, but their integrity is restored only if their central and peripheral cut ends are brought sufficiently closely to each other. The regenerating axons grow along a path within the sheath of Schwann of the degenerating peripheral segment, and subsequently form here mature, functioning nerve fibers. Axons which fail to enter the sheath of Schwann very quickly undergo degeneration [5, 6]. The sheath of Schwann is an essential medium for the axons, which is concerned in the metabolism of the nerve fiber and forms the myelin sheath. When there is a gap or defect in the nerve trunk, the process of regeneration is made more difficult, the regenerating axons are lost in the surrounding tissues and they fail to reach the peripheral segment. Numerous attempts to provide a suitable path for the newly formed axons have not given the desired results. This problem requires further experimental investigation.

We considered that a segment of nerve, transplanted into a defect, would not simply be a path along which the axons could grow, but would also take part in the formation of mature nerve fibers, would undergo Wallerian degeneration, and would create the necessary conditions for the perfect regeneration of a nerve at the site of the defect. Such conditions, of course, could only be obtained by the use of fresh nerves as material for the graft.

Segments of nerve trunks, when transplated in animals, are known to undergo Wallerian degeneration [2, 7]. Autotransplantation of the nerve gives the highest percentage of lasting preservation; homotransplantation of nerves ends in failure in the overwhelming majority of cases, for homografts last for only 18 days in a state of Wallerian degeneration [7], after which their Schwann cells die and are absorbed. This phenomenon of death of the homograft is explained by the incompatibility of the graft and the host; the body reacts to the homograft as to an antigen. When using homografts it is therefore necessary to find effective ways of overcoming these manifestations of incompatibility.

This biological incompatibility of the tissues of the donor and the recipient has been overcome by decreasing the antigenic properties of the graft, and also by weakening the reactivity of the host. Research in recent years has shown that the immune reactions of the body depend on the state of the central nervous system. By producing inhibition in the central nervous system as the result of induction of sleep by drugs, M. I. Efimov and Sh. V. Musina [5] successfully obtained the survival of a skin homograft, and Yu. M. Gudzovskaya [3] – survival of a nerve homograft.

Irradiation of an animal with x-rays has also been shown to lead to inhibition of tissue processes.

A. A. Braun and K. M. Akylbekov [1] obtained true survival of skin homografts in rats in 40% of cases, by preliminary irradiation of the animals with a certain dose of x-rays.

In this way we decided to attempt to overcome the intolerance of the recipient towards a nerve graft, by irradiation of the host with x-rays.

METHODS

Experiments were carried out on rabbits. For 14 days before operation, the rabbits were given whole-body irradiation from the ventral aspect, under the following conditions: 180 kv, 20 ma, filter – Cu 0.5 mm, skin-focus distance 60 cm, dose rate 19 r/min, total dose 500 r. Two weeks after irradiation, under general anesthesia, a segment of the sciatic nerve, 1-1.5 cm long, was excised from the rabbits on the right side. The defect thus produced was closed by means of a homograft of suitable size. The rabbits of a control group underwent the operation without preliminary irradiation.

Rabbits were sacrificed at three times: 14-20 days, 30-35 days, and 2 months after operation. The nerves of the limb on which the operation had been performed were excised, fixed in 90% alcohol, impregnated with silver by Cajal's method, and embedded in paraffin wax; serial sections were then studied.

RESULTS

In the control group of animals (4 rabbits) one month after operation, in general no growth of axons

through the graft could be observed. All the grafts consisted of empty sheaths of Schwann; solitary nerve fibers were encountered mainly in the peripheral parts of the graft.

In the peripheral segment of the nerve, nerve fibers were completely absent; this also consisted of empty sheaths of Schwann.

In the experimental group of animals, sacrificed 14-20 days after operation (3 rabbits) we observed the following. In one rabbit the histological picture showed no difference from that described in the control animals i.e., the whole graft appeared empty; solitary nerve fibers were encountered only in the peripheral areas of the graft. The peripheral segment was completely without nerve fibers. In the second rabbit, very fine nerve fibers were found throughout the thickness of the graft, few in number, and in places pursuing a highly irregular course. A larger number of fibers was observed in the peripheral parts of the graft. The bulk of the sheath of Schwann, however, contained no nerve fibers. Nerve fibers were also absent from the peripheral segment. In the third animal of this group, at this time, the whole graft appeared to be filled with nerve fibers, which were quite thickly packed and followed a parallel course. In this case axons had grown through the sheath of Schwann, and in places, nuclei of the Schwann cells could be seen. No axons were found in the peripheral segment of the nerve in all three rabbits.

In the irradiated animals that were sacrificed 30-35 days after operation (5 rabbits) the following microscopic changes were observed. In all the animals of this group nerve fibers were growing through most of the graft; in 2 rabbits, however, there were only a few nerve fibers, quite a long way apart from each other, in the whole thickness of the graft. In the 3 remaining rabbits nerve fibers were fairly closely packed together in the graft, and they were parallel to each other.

Nuclei of Schwann cells could be clearly seen among the nerve fibers in 2 animals. In one animal the nerve fibers attained a great thickness and had a very irregular course, so that they appeared interrupted in the section. In one case it could be seen that small trunks of nerve fibers left the graft and went in the direction of the muscles alongside it. No regenerating axons could yet be seen in the peripheral segment of the nerve in all 5 rabbits.

At a later period – 2 months after operation (6 rabbits) – in one rabbits there was no general regeneration of nerve fibers, but only solitary nerve fibers were encountered in the peripheral areas of the graft. In another animal a considerable number of nerve fibers was seen in the peripheral parts of the graft, and in the central part of the graft only few fibers were seen, having a very irregular course. In the remaining 4 animals of this group, large numbers of nerve fibers had grown through the graft (Fig. 1). In transverse sections it could be seen that axons were embedded

Fig. 1. Longitudinal section in the region of a nerve homograft 2 months after transplantation in a rabbit exposed to preliminary irradiation. Nerve fibers, nuclei of Schwann cells, and blood vessels are clearly visible in the graft. Microphotograph. Magnification: ocular 7 ×, objective 40 ×. Cajal's silver impregnation.

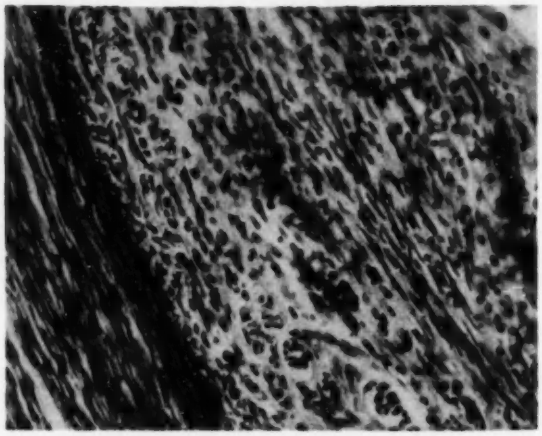


Fig. 2. Transverse section in the region of a nerve homograft 2 months after transplantation in a rabbit exposed to preliminary irradiation. Axons are seen, singly and a few at a time, embedded in the sheath of Schwann. Microphotograph. Magnification: ocular 7 ×, objective 40 ×. Cajal's silver impregnation.

singly or, more often, a few at a time in the sheath of Schwann (Fig. 2). Blood vessels with blood cells and nuclei of Schwann cells were clearly visible in places in the grafts. It was readily seen that nerve fibers, after passing through the region of the scar, grew from the graft into the peripheral segment. Here, in the peripheral segment of the nerve axons were now present, but the nerve fibers were fewer and further apart than in the graft, although they had the usual appearance and were parallel to each other (Fig. 3).

It follows from these findings that preliminary whole-body irradiation of a rabbit with a dose of 500 r

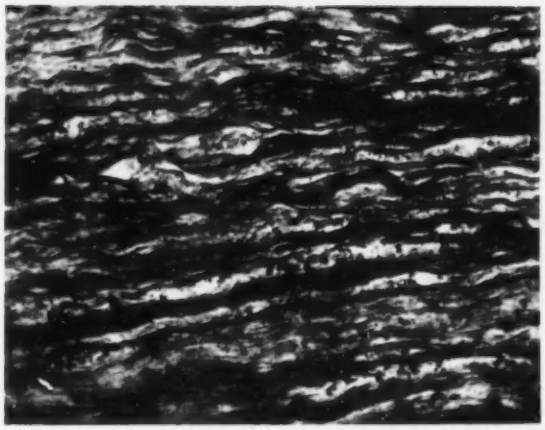


Fig. 3. Longitudinal section through the peripheral segment of a nerve 2 months after operation. The nerve fibers have the usual appearance and are arranged parallel to each other, but a considerable distance apart. Microphotograph. Magnification: ocular 7×, objective 40×. Cajal's silver impregnation.

of x-rays lowers the reactive properties of the animal and thereby protects the homograft, i.e., its Schwann cells, from death; this thus creates favorable conditions for nerve fibers to grow out from the central segment of the nerve, through the graft, into the peripheral end. With a defect of 1-1.5 cm and a nerve homograft of suitable length, regeneration of nerve fibers in the peripheral segment is observed 2 months after operation.

SUMMARY

The author studied the regeneration of the nerves at defects. A nerve homotransplant was used to replace the defect, the necrosis of the graft being prevented by preliminary X-ray irradiation of the host animal in a dose of 500 r. As a result, a few axons could be seen in the transplant 20 days after implantation; in a month's time this number had greatly increased, while in 2 months there were even more axons present, and they were present within the Sheath of Schwann. During this period, a small number of axons appeared in the peripheral section of the nerve.

LITERATURE CITED

[1] A. A. Bruan and K. M. Akylbekov, Doklady Akad. Nauk SSSR 95, 6 (1954).

[2] E. A. Gromova, Trudy Akad. med. nauk SSSR 19, 1 (1952).

[3] Yu. M. Gudzovskaya, Doklady Akad. Nauk SSSR 100, 1 (1955).

[4] B. S. Doinikov, Fifth Session of the Neurosurgical Committee [in Russian] (1940).

[5] M. I. Efimov and Sh. V. Musina, Doklady Akad. Nauk SSSR 47, 1, (1951).

[6] B. I. Lavrent'ev, Byull. éksptl. biol. i med. 18, 12 (6) (1944).

[7] A. Ingebrigsten, Chir. (Lyon) 13, 5 (1955).

THE EFFECT OF AUTONOMIC STIMULATORS ON CELL DIVISION IN THE CORNEAL EPITHELIUM

E. S. Tsareva

Department of Histology (Head – Prof. V. A. Ravvin) of the Stalino Medical Institute

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny
Vol. 49, No. 1, pp. 115-117, January, 1960

Original article submitted June 29, 1959

In recent years much research has been carried out on the relationship between mitotic activity and the state of the nervous system. As a result of this research, many facts have been obtained which point to the importance of the central nervous system in the regulation of cell division [1, 2, 4, 7, 9, 10, 11, etc]. So far as the influence of the autonomic division of the nervous system on the process of formation of cells by mitosis is concerned, it is only recently that work on this problem has begun [1, 3, 5, etc].

In the present research, we investigated the mitotic activity in the corneal epithelium in different functional states of the autonomic nervous system. Changes in the tone of the autonomic nervous system were brought about by administration of various autonomotropic drugs.

METHODS

As a sympatheticomimetic drug we used adrenalin, and to lower the tone of the sympathetic nervous system, we used ergotin, which lowers the sensitivity of the animal to adrenalin. In order to study the influence of the parasympathetic tropic drugs on mitotic activity we used prostigmin, which promotes the accumulation of acetylcholine in the tissues; as a result of this, the tone of the parasympathetic nervous system is raised. As a cholinolytic drug we used atropine. All these various autonomotropic drugs were injected subcutaneously 1-3 hours before decapitation of the animal, in a dose of 0.1 mg/kg body weight, apart from atropine, which was injected in a dose of 0.6 mg/kg body weight.

Experiments were carried out on sexually mature white mice (males) of about the same age. The animals were decapitated always at the same time of day – 12 hr, 30 min. Total preparations of the cornea were obtained and stained with Carazi's hematoxylin. When the dividing cells were being counted, we took into consideration all the stages of cell division. As an index of mitotic activity, we adopted the number of mitoses in the whole cornea, multiplied by 100 and divided by the number of fields of vision. The figures obtained were treated statistically.

For the experiments we used 125 animals. In 65 of these we studied the influence of sympatheticotropic substances, including experiments with adrenalin on 40 mice (20 experimental and 20 control) and experiments with ergotin and 25 animals (15 experimental and 10 control). As regards the study of the influence of the parasympatheticotorpic drugs on the mitotic activity of the corneal epithelium, in experiments on 20 mice the parasympathetic nervous system was stimulated (12 experimental and 8 control animals) and in experiments on 40 animals the influence of the parasympathetic nervous system was depressed (25 experimental and 15 control animals).

RESULTS

According to our findings, in the experiments in which adrenalin was given, a depression of mitotic activity was observed; this was shown by a sharp reduction in the early stages of division in the corneas of the experimental mice. On the average, the decrease in the number of mitoses by comparison with the controls was 67% (Table 1).

It must be mentioned that these figures are in complete agreement with the results of several workers [6, 8, etc.], who have studied the effect of adrenalin on mitosis.

In the experiments in which the sympathetic nervous system was depresed, we obtained quite different results. As is shown by the figures given in Table 1, the injection of ergotin not only did not depress mitotic activity but, on the contrary, it resulted in an increase in the number of mitoses in the corneas of the experimental mice; this increase on the average amounted to 39%. The increase in the absolute number of mitoses in the corneas of the experimental mice was mainly due to the early phases of division – the prophase and metaphase – and it may thus be considered that in the experiments in which the tone of the sympathetic nervous system was lowered, the mitotic activity was increased. Consequently, according to the results obtained in the experiments with administration of sympatheticotropic drugs, an increase in the tone of the

TABLE 1 Experiments in which Sympatheticotropic Drugs were Injected

Experimental series	Subgroup	Number of animals (n)	Mean number of mitoses, M	Difference between C and E: in absolute units ($M_E - M_C$)	Difference between C and E: in % $\frac{(ME - MC)}{MC} \cdot 100\%$	Criterion of significance of the difference between the arithmetic means, $t = \frac{(M_E - M_C)}{\sqrt{m_o^2 + m_c^2}}$
I (adrenalin)	Control (C)	20	64	43	67	16.9
	Exp. (E)	20	21			
II (ergotin)	Control (C)	10	70	27	39	6.43
	Exp. (E)	15	97			

TABLE 2 Experiments in which Parasympatheticotropic Drugs were Injected

Experimental series	Subgroup	Number of animals (n)	Mean number of mitoses, M	Difference between C and E: in absolute units ($M_E - M_C$)	Difference between C and E: in % $\frac{(ME - MC) \cdot 100\%}{MC}$	Criterion of significance of the difference between the arithmetic means, $t = \frac{(M_E - M_C)}{\sqrt{m_o^2 + m_c^2}}$
III (prostigmin)	Control (C)	8	70	22	31	5.52
	Exp. (E)	12	92			
IV (atropine)	Control (C)	15	77	19	25	3.78
	Exp. (E)	25	58			

sympathetic nervous system in the body, brought about by injections of adrenalin, caused a depression of mitotic activity, but a temporary depression of the influence of the sympathetic nervous system (by injection of ergotin) resulted in the stimulation of mitotic activity. It may hence by concluded that the sympathtic nervous system evidently exerts an inhibitory influence on the process of cell division.

The study of the influence of the parasympathetic nervous system in the regulation of cell division was the object of the next 2 series of experiments. In one of these, in response to the injection of prostigmin, an increase in mitotic activity was observed in the experimental mice — on the average by 31% (Table 2).

In the experiments in which atropine was injected, on the other hand, the total number of mitoses was reduced (on the average by 25%), and there was also a fall in the number of early phases of mitosis.

When comparing the results obtained in the different series of experiments, it should be noted that during exclusion of the sympathetic nervous system and during excitation of the parasympathetic nervous system analogous changes were observed in the corneal epithelium: the number of cells undergoing mitosis was increased. During an elevation of the tone of the sympathetic nervous system, as during lowering of the tone of the parasympathetic nervous system, on the other hand, there was a depression of mitotic activity.

These findings are evidence that, firstly, the level of mitotic activity of the corneal epithelium depends on the functional state of the autonomic division of the nervous system; the sympathetic nervous system inhibits the process of cell division and the parasympathetic nervous system has a stimulatory effect on this process, i.e., in the regulation of mitotic activity they behave as antagonists.

SUMMARY

The paper deals with investigations of the mitotic activity in the corneal epithelium of white mice in different functional states of the autonomic nervous system. The latter was attained by the injection of the following autonomotropic substances: adrenalin, ergotin, prostigmin and atropine. Upon injection of adrenalin and atropine the mitotic activity level dropped, whereas the administration of ergotin and prostigmin increased the mitotic activity. The data obtained testify to the fact that the sympathetic nervous system exerts an inhibitory influence on the mitotic activity of the corneal epithelium, while the parasympathetic nervous system has a stimulating action.

LITERATURE CITED

[1] I. A. Alov, Arkh. anat., gistol. i embriol. 32, 4, 43-46 (1955).

[2] S. Ya. Zalkind, Doklady Akad. nauk SSSR 99, 6, 1011 (1954).

[3] M. K. Zakharov, Arkh. anat., gistol. i embriol. 32, 4, 47-53 (1955).

[4] V. V. Kozlov, Doklady Akad. Nauk SSSR 99, 2, 317 (1954).

[5] Kh. Ya. Puzhaka, Author's abstract of dissertation [in Russian] (Riga, 1959).

[6] A. K. Ryabukha, Doklady Akad. Nauk SSSR 104, 4, 642-645 (1955).

[7] G. S. Strelin, L. B. Bychkovskaya, and V. V. Kozlov, Doklady Akad. Nauk. SSSR 99, 1, 165 (1954).

[8] L. V. Suvorova, Author's abstract of dissertation [in Russian] (Leningrad, 1955).

[9] L. A. Utkin, Dissertation [in Russian] (Moscow, 1951).

[10] O. Cheng, Author's abstract of dissertation [in Russian] (Leningrad, 1955).

[11] J. Friedenwald and W. Buschke, Am. J. Physiol. 141, 5, 689-694 (1944).

THE STATE OF THE LYMPHATICS DURING DISTURBANCE OF THE VENOUS RETURN FROM THE LIMBS IN DOGS

A. G. Fedorova

Department of Normal Anatomy (Head – Corresponding Member AMN SSSR Prof. B. A. Dolgo-Saburov) of the S. M. Kirov Order of Lenin Military Medical Academy, Leningrad

(Presented by AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny
Vol. 49, No. 1, pp. 118 , January, 1960

Original article submitted February 28, 1959

The anatomy of the parietal and visceral lymphatic vessels has now been studied in some detail in respect to all the organs and tissues of man and certain animals [2, 3, 6, 8, 11, 12, 15, and many others]. The role of the lymphatic system in metabolism and hemopoiesis and its participation in pathological processes have also been demonstrated.

Many authors have investigated the collateral circulation of lymph [1, 4, 5, 6, 7, 9, 10, 13, 14, and many others].

Considerable attention has been devoted to lymphatico-venous anastomoses. The majority of workers categorically deny the existence of such structures. Nevertheless, V. Kh. Frauchi [19] demonstrated the existence of a direct connection between the thoracic duct and the veins of the inferior vena cava system in the region of the lower thoracic and the lumbar vertebrae. The presence of a close connection between the lymphatic and blood systems has been known since the end of the last century. I. A. Notkin [13], for instance, in experiments in which he ligated the portal vein in dogs, found dilatation of the mesenteric lymphatic vessels, and V. N. Popov [16] drew attention to the fact that ligation of veins results in an increase in the volume of lymph. After ligation of arteries, bleeding, and stimulation of the vagus nerve, V. N. Popov observed the converse effect.

V. V. Stavskaya [14] ligated various main venous trunks (femoral, external iliac, subclavian, and inferior vena cava) in acute experiments on dogs, and investigated the parietal lymphatic vessels. She found an abnormally large number of lymphatic vessels, starting 5-10 minutes after ligation of the veins. M. S. Yaroshetskaya [21] observed a similar picture in chronic experiments in which the external iliac vein was ligated in dogs. M. V. Shevelev [20] ligated the portal vein in cats and observed enlargement of the mesenteric lymph glands and dilatation of the lymphatic vessels in the mesentery of the large and small intestines.

Recently, V. Petrovskii and his co-workers [15] have shown by means of physiological methods of investigation that "the tone of the lymphatic vessels is determined by the activity of their center, which is a part of the vasomotor center. A change in the tone of the lymphatic vessels occurs simultaneously with a change in the tone of the blood vessels". Similar phenomena were observed after the stimulation of zones known to be reflexogenic for blood vessels (regions of the aorta, the pulmonary vessels, the carotid sinuses, the inferior vena cava and the portal, mesenteric, splenic, and renal veins). Besides reflexes from the blood vessels to the lymphatics these authors also established reflexes in the opposite direction. An increase of pressure in the receptaculum chyli and the thoracic duct, for instance, produced a reflex lowering of the arterial pressure.

Our attention was drawn to the lymphatic system after we had found that when the venous return was disturbed in chronic experiments, dilatation and partial varicosity of not only the veins but also the visceral arteries of medium and small caliber ensued.

This fact led us to make a special investigation of the reaction of the lymphatic vessels under these circumstances, in order to obtain a complete picture of the state of the vascular system as a whole after excision of the main veins of the limbs. The results obtained (because of the small number of animals investigated) cannot, of course, claim to provide an exhaustive answer to the problem. They are merely the beginning of the study of this important problem.

METHOD

Investigations were made of the lymphatic vessels of the fore and hind limbs of 6 dogs at various times after operation – two were studied 14 days after excision of the main veins of the limbs, two 45 days after and two 90 days after operation. Injection of the parietal lymphatic vessels of the experimental and control animals gave no particular difficulty. Injection of the visceral lymphatics was a different matter. In this case satisfactory injection could only be achieved in the network

of lymphatic vessels of the fascia and tendons of certain muscles (the triceps brachii and the triceps surae).

RESULTS

The preparations obtained showed that excision of the main veins leads to disturbance of the lymphatics as well as of the blood vessels.

In the animals two weeks after operation, an increase was observed in the number and caliber of the parietal lymphatic vessels (Fig. 1). The visceral lymphatic vessels of the muscle fascia and tendons were also sharply dilated (Fig. 2). In the preparations obtained from animals which survived 1½ months after excision of the main veins, the caliber of the parietal lymphatic vessels remained enlarged as before, but their number had decreased approximately to that seen in the control limbs. The visceral lymphatic vessels were appreciably dilated, but this dilatation was less pronounced than in the animal 2 weeks after operation.

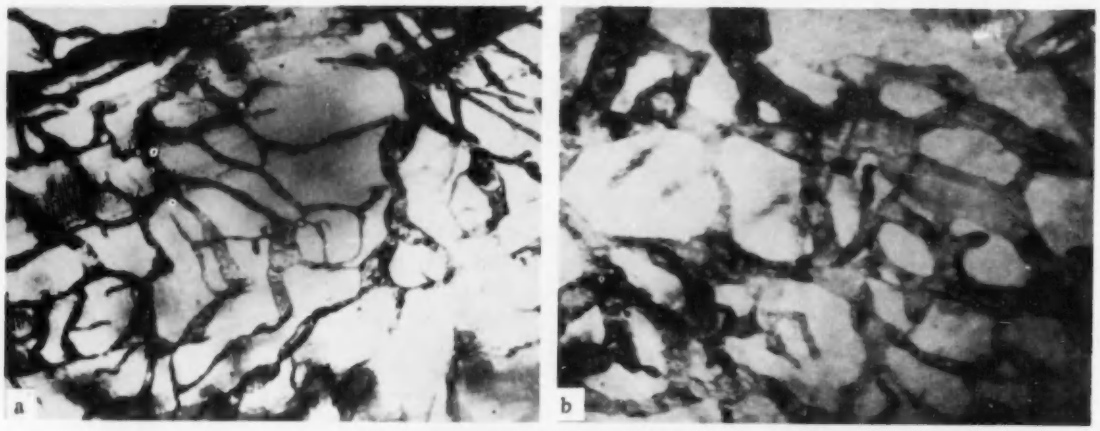


Fig. 1. Superficial parietal lymphatic vessels of the fore limbs of dogs on the 14th day after excision of the main veins. a) Control; b) experiment.

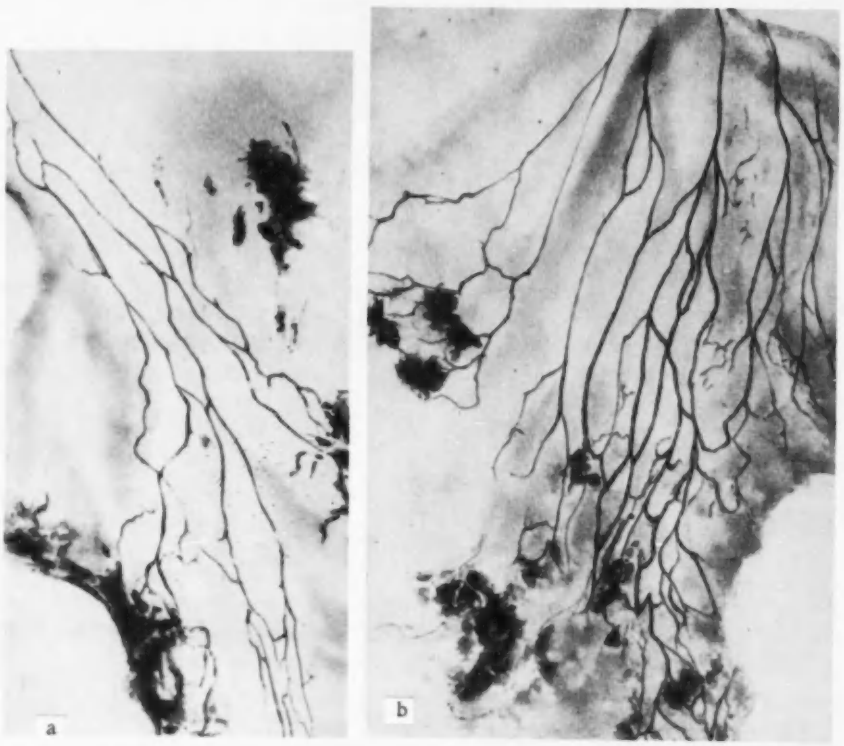


Fig. 2. Visceral network of lymphatic vessels of the tendo Achillis. a) Experiment; b) control.

In the dogs which survived 3 months after operation, we were unable to find any appreciable morphological changes whatsoever in the lymphatic system of the limbs.

The reorganization of the lymphatic system which we observed in the first 1½-2 months after excision of the main veins must be regarded as a regular phenomenon. At this period, as we know, stimuli are constantly in action. Foremost among these is the raised blood pressure in the distal divisions of the limb. This factor, as shown by V. Petrovskii and his co-workers, causes reflex dilatation of the lymphatic capillaries. With the cessation of the action of these stimuli, the caliber of the lymphatic vessels gradually returns to normal, as we found in our own experiments.

In the presence of disturbance of the venous return, it is absolutely inadequate to investigate the venous system alone. In order to evaluate accurately the conditions under which the tissues function, it is of the utmost importance to direct attention to the arterial and lymphatic vessels also, for the vascular system functions as a single entity, not only when the normal conditions of existance of the animal apply, but also when the blood flow is disturbed.

SUMMARY

After excision of the great of extremities in dogs, there is observed not only the development of parietal collateral venous paths and dilatation of the visceral blood vessel network (arteries and veins), but also considerable changes in the lymphatic vessels. The latter were considerably dilatated during the first 14-45 days after the experimental surgical intervention. Both parietal and visceral lymphatic vessels are subject to dilatation. At the end of the third month of the postoperative period the changes observed earlier begin to gradually disappear and the lymph vessels of the limb under experiment show no morphological differences from the control limb.

LITERATURE CITED

[1] R. A. Bardina, Anatomy of the Visceral Vessels [in Russian] (Leningrad, 1948) p. 262.

[2] V. V. Ginzburg, Paths of the Outflow of Lymph from the Muscles of the Lower Limb [in Russian] Doctorate dissertation (Samarkand, 1944).

[3] V. P. Golev, Transactions of the Fifth All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian] (Leningrad, 1951) p. 144.

[4] D. E. Gol'dshtein, Trudy Kazansk. nauch.-issled. inst. ortopedii i vosstano-vitel'noi khirurgii 3, 297 (1949).

[5] D. A. Zhdanov, General Anatomy and Physiology of the Lymphatic System [in Russian] (Leningrad, 1952).

[6] N. L. Zotova, Anatomy of the Visceral Vessels [in Russian] (Leningrad, 1948) p. 271.

[7] A. I. Ivanova, Anatomy of the Visceral Vessels (Leningrad, 1948) p. 267.

[8] G. M. Iosifov, The Lymphatic System in Man [in Russian] (Tomsk, 1914).

[9] K. S. Malinovskii, The Lymphatic System of the Lower Limb [in Russian] Dissertation (Vitebsk, 1906).

[10] N. N. Mirolyubov, Anatomy of the Visceral Vessels [in Russian] (Leningrad, 1948) p. 264.

[11] N. N. Mirolyubov, Collected Transactions of the Irkutsk Medical Institute [in Russian] (1951) p. 78.

[12] V. N. Nadezhdin, Investigation of the Anatomy of the Lymphatic System of the Lower Limb in Man [in Russian] Author's abstract of dissertation (Leningrad, 1957).

[13] I. A. Notkin, On the Origin of Ascites [in Russian] (Kiev, 1890).

[14] V. V. Stavskaya, Anatomy of the Visceral Vessels [in Russian] (Leningrad, 1948) p. 270.

[15] V. Petrovskii, Med. Rabotnik (July 20, 1956), p. 3.

[16] V. N. Popov, The Drainage of Lymph [in Russian] (Moscow, 1893).

[17] M. G. Prives, Collected Papers on Field and General Surgery [in Russian] (Leningrad, 1944) p. 103.

[18] V. V. Fedyai, Anatomy of the Lymphatic Vessels and Glands [in Russian] (Gor'kii, 1942) p. 197.

[19] V. Kh. Frauchi, Khirurgiya 11, 12 (1948).

[20] M. V. Shevelev, Collection of Abstracts of Scientific Papers of the Military Medical Academy for the Period 1953-1955 [in Russian] (Leningrad, 1957) p. 32.

[21] M. S. Yaroshetskaya, Anatomy of the Visceral Vessels [in Russian] (Leningrad, 1948) p. 268.

THE SPINAL SENSORY COMPONENT OF THE GANGLIA OF THE SYMPATHETIC NERVOUS SYSTEM

N. K. Landshman

Department of Histology and Embryology (Head – Corresponding Member AN SSSR Prof. G. K. Khrushchev, Director of Research – Prof. T. A. Grigor'eva) of the Second Moscow Medical Institute (Director – Docent M. G. Sirotkina)

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 122-125, January, 1960

Original article submitted July 29, 1959

The sensory innervation of the internal organs by the peripheral processes of neurones whose bodies lie in the spinal ganglia is now accepted beyond doubt. An important part in the discovery of the spinal sensory innervation of the internal organs was played by B. I. Lavrent'ev and his pupils, who proved experimentally the spinal sensory innervation of the heart [16], the blood vessels [3], the adrenals [9], and the urinary bladder [17]. Thd spinal sensory innervation of all the internal organs [6] and blood vessels [5, 8] was subsequently demonstrated.*

The spinal sensory fibers pass into the sympathetic trunk from the spinal nerves, via the rami communicantes, and then pass both caudally and cranially along the sympathetic trunk [2, 7, 20, 21]. Therefore, through any given segment of the sympathetic nervous system, spinal sensory fibers pass from the whole series of intervertebral ganglia. For instance, through the greater splanchnic nerve and the celiac ganglion pass spinal sensory fibers from the lower cervical [14] and thoracic [7, 10, 19] intervertebral ganglia, the bulk of them being derived, according to these authors, from the spinal ganglia $D_8 - D_{10}$. Through the stellate ganglion pass spinal sensory fibers from intervertebral ganglion $C_5 - L_2$ [13].

We have been unable to find in the literature any reports giving an accurate quantitative description of the sources of the spinal sensory fibers passing rhrough the individual sympathetic ganglia. The aim of the present research was to make quantitative analysis of the segmental distribution of the spinal sensory component of the celiac, stellate, and superior cervical sympathetic ganglia.

METHODS

In 3 series of experiments the celiac, stellate, and superior cervical sympathetic ganglia were extirpated unilaterally or bilaterally in adult cats. In each series the extirpation was done unilaterally in 3 animals and bilaterally in 2-5 animals. Control operations were also performed (under the same conditions, but the corresponding ganglia were not removed). The animals were killed with ether 8-19 days after operation. With unilateral extirpation of the sympathetic ganglia, all the cervical, thoracic, and lumbar intervertebral ganglia on both sides were taken for examination, and with bilateral extirpation and after the control operations, the same intervertebral ganglia from one side of the body of the animal were taken.

In addition, the spinal ganglia of clinically healthy, unoperated animals were studied. The ganglia were fixed in alcohol and embedded in paraffin wax. Serial sections of the intervertebral ganglia were cut to a thickness of 8 μ, every tenth section being retained for examination. The sections were stained with toluidine blue, and also with gallocyanine.

In extirpating any sympathetic ganglion, naturally all the spinal sensory fibers passing through it were divided. In consequence of this, retrograde changes arose in the corresponding neurones of the intervertebral ganglion (Fig. 1, a and b). By employing this phenomenon we were able to determine both the localization of the neurones whose processes passed through the extirpated sympathetic ganglion, and the number of these neurones in all the cervical, thoracic, and lumbar intervertebral ganglia. The latter was done by counting the number of nerve cells undergoing retrograde changes in the serial sections of all the intervertebral ganglia taken from the animals after unilateral extirpation of the sympathetic ganglia. We estimated the total number of neurones undergoing retrograde changes separately in the intervertebral ganglia on right and left sides. Then, also separately in the ganglia of the right and left sides, we calculated the percentage content of these neurones in each interverte-

* Questions relating to the role of the Dogiel Type 2 cells are not discussed in this paper.

bral ganglion. Mean values were deduced for all the series of experiments.

RESULTS

After extirpation of the celiac ganglion of the solar plexus, we found retrograde changes in neurones in all the cerebrospinal ganglia at the level of the segments C_5-L_4. This meant that through the celiac ganglion of the solar plexus passed processes from neurones whose bodies were situated in intervertebral ganglia C_5-L_4 on the ipsilateral side of the animal's body. The bulk of these neurones (89.6%) were concentrated in the intervertebral ganglia D_7-L_3 (Fig. 2). Through the celiac ganglion also passed processes from nerve cells of intervertebral ganglia C_6-L_4 of the contralateral side; these cells, however, were only one fifth as frequent as in the cerebrospinal ganglia after removal of the ipsilateral celiac ganglion. These nerve cells were also distributed irregularly in the intervertebral ganglia on the contralateral side.

Through the stellate ganglion passed processes of neurones whose bodies lay in intervertebral ganglia C_2-L_4 on the ipsilateral side of the body. These neurones were mainly in intervertebral ganglia C_6-D_5 (74% Fig. 3). Neurones of spinal ganglia C_2-L_3 of the contralateral side also sent their processes to the periphery through the stellate ganglion, but such neurones were from 2 to 2.6 times less frequent than in the cerebrospinal ganglia of the ipsilateral side, although the gradient of their distribution among the spinal ganglia of the contralateral side was similar to that of the ipsilateral side.

Through the superior cervical ganglion passed processes of nerve cells in intervertebral ganglia C_2-D_4 of both ipsilateral and contralateral sides. The numbers of these nerve cells, however, were comparatively small. For instance, whereas through the celiac ganglion passed 913-1204 processes of spinal sensory neurones from the ipsilateral side, and through the stellate ganglion 450-714 processes, through the superior cervical sympathetic ganglion only 30-35 processes passed from the intervertebral ganglia on the ipsilateral side. In intervertebral ganglia C_2 and C_3 the number of nerve cells sending processes through the superior cervical sympathetic ganglion was as a rule larger (5-7 neurones in each ganglion) than in ganglia C_4-D_4 (2-4 neurones in each ganglion).

In the intervertebral ganglia of the control and clinically healthy animals, neurones showing retrograde changes were encountered extremely rarely; only in occasional ganglia did we find solitary nerve cells with signs of retrograde changes.

D. M. Golub [1] showed that through any given ganglion of the sympathetic nervous system pass processes from neurones of a large number of intervertebral ganglia; the bulk of these neurones, however, lies in intervertebral ganglia situated close to this particular area of the sympathetic nervous system, and only a

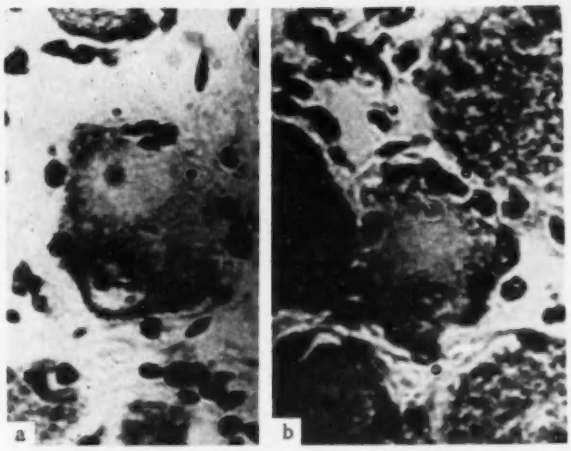


Fig. 1. Retrograde changes in a cell in cerebrospinal ganglion D_2 on the right side 12 days after extirpation of the right stellate ganglion (a). Retrograde changes in a cell in cerebrospinal ganglion C_3 on the right side 16 days after extirpation of the right superior cervical sympathetic ganglion (b). Alcohol. Toluidine blue. Magnification: ocular 10 ×, objective 40 ×.

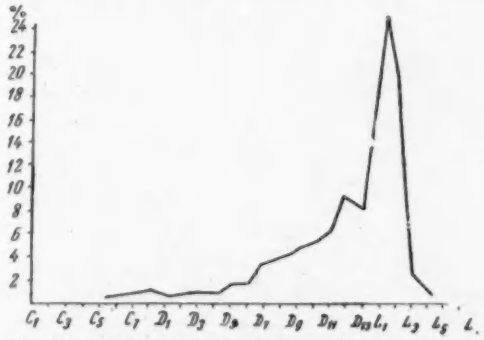


Fig. 2. Gradient of distribution of the sources of the spinal sensory fibers passing through the celiac ganglion.

small number of neurones is derived from more distant ganglia. Our findings confirmed this view and provided more accurate quantitative details. Many reports speak of the same multisegmental principle of the sensory innervation of the internal organs and blood vessels [4, 5, 11, 13, etc.].

It must be considered that this multisegmental principle arose in the course of evolution. In Amphioxus the visceral branches leave the posterior roots metamerically; the visceral branches proceed equally metamerically in the Cyclostomata, and in these, moreover, the ganglia of the sympathetic trunk are still without longitudinal interconnections; only in the bony fish do longitudinal connections appear between the sympathetic ganglia [18, 22]. In Amphibia, the longitudinal connections of the sympathetic trunk are more highly developed, but a large number of visceral branches still follow a metameric path; in birds there are far fewer of these metameric visceral branches, and in mammals

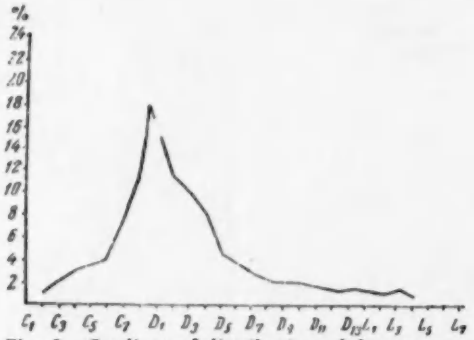


Fig. 3. Gradient of distribution of the sources of the spinal sensory fibers passing through the stellate ganglion.

and in man they are concentrated to the greatest degree in individual trunks [15].

The appearance of this multisegmental principle of the spinal sensory innervation of the internal organs and blood vessels in the course of evolution may be associated with the necessity for improving the nervous connections between the internal organs, and also between the internal organs and the central nervous system. The large number of connections permits the nervous system to obtain better correlation of the work of the internal organs, and to switch the maximum load rapidly from some organs to others. This promotes greater stability of the animal in the constantly changing conditions of the external environment.

The exteroceptive innervation of the skin and the proprioceptive innervation of the muscles have preserved their strictly segmental character, for the first enables localized perception of external stimuli, necessary for the organization of a purposive response reaction, to be obtained, and the second provides information about the position of parts of the body in space, which is important for the accuracy and precision of locomotor acts.

The different trends of evolution of the sensory innervation of the internal organs, on the one hand, and of the exteroceptive innervation of the skin and the proprioceptive innervation of the muscles on the other hand, have thus been conditioned by different principles of the structural organization of these afferent systems.

SUMMARY

The author studied the sources of the spinal sensory fibers of the celiac, stellate and superior cervical sympathetic ganglia.

These ganglia were removed in cats and in 8-19 days all the cervical thoracic and lumbar spinal ganglia were studied in serial sections to reveal retrograde changes in the neurones.

It was established that the neuron processes of the spinal ganglia C_5 and L_1 of the ipsilateral side pass through the celiac ganglion. The bulk of these neuroneslies in the spinal ganglia D_7-L_3. The neurone processes of the spinal ganglia C_2-L_4 pass through the stellate ganglion, but mainly $-C_7-D_5$; the neurone processes of the spinal ganglia C_2-D_4 pass through the superior cervical sympathetic ganglion.

The neurone processes of the spinal ganglia of the contralateral side pass through all the sympathetic ganglia mentioned.

LITERATURE CITED

[1] D. M. Golub, Problems of the Morphology of the Peripheral Nervous System [in Russian] (Minsk, 1953) Vol. 2, p. 5.

[2] D. M. Golub and B. M. Kichina, Problems of the Morphology of the Peripheral Nervous System [in Russian] (Minsk, 1953) Vol. 3, p. 11.

[3] T. A. Grigor'eva, Morphology of the Sensory Innervation of the Internal Organs [in Russian] (Moscow, 1947) p. 84.

[4] T. A. Grigor'eva, Uspekhi sovremennoi biol. 28, 1, 150 (1949).

[5] T. A. Grigor'eva, Innervation of the Blood Vessels [in Russian] (Moscow, 1954).

[6] T. A. Grigor'eva, Arkh. anat. gistol. i embriol. 36, 3, 11 (1959).

[7] A. S. Gusev, The Structure of the Splanchnic Nerves [in Russian] Candidate's dissertation (Leningrad, 1954).

[8] B. A. Dolgo-Saburov, Innervation of the Veins [in Russian] (Leningrad, 1958).

[9] V. I. Il'ina, Morphology of the Sensory Innervation of the Internal Organs [in Russian] (Moscow, 1947) p. 135.

[10] B. M. Kichina, Problems of the Morphology of the Peripheral Nervous System [in Russian] (Minsk, 1958) Vol 4, p. 29.

[11] E. M. Krokhina. The Sensory Innervation of the Large Intestine in Mammals [in Russian] Candidate's dissertation (Moscow, 1947).

[12] B.I. Lavrent'ev, Zhur. obshchei biol. 4 4, 232 (1943).

[13] V. F. Lashkov, Arkh. anat., gistol. i. embriol. 31 (1952).

[14] P. I. Lobko, Proceedings of the Sixth All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian] (Khar'kov, 1958) p. 407.

[15] G. A. Orlov, The Surgical Anatomy of the Sympathetic Trunk [in Russian] (Arkhangel'sk, 1946).

[16] E. K. Plechkova, The Sensory Innervation of the Internal Organs [in Russian] (Moscow, 1947) p. 46.

[17] E. K. Plechkova, The Sensory Innervation of the Internal Organs [in Russian] (Moscow, 1947) p. 167.

[18] B. M. Sokolov, A General Study of the Ganglia [in Russian] (Perm', 1943).

[19] F. B. Kheinman, Problems of the Morphology of the Peripheral Nervous System [in Russian] (Minsk, 1958) Vol. 4, p. 41.

[20] J. Fischer, Anat. Ans. 26, 388 (1905).

[21] W. H. Gaskell, J. Physiol. 7, 1 (1886).

[22] C. U. A. Kappers and others, The Comparative Anatomy of the Nervous System of Vertebrates, Including man (New York, 1936).

TECHNIQUE OF EMBEDDING DRY ANATOMICAL AND SPECIMENS IN ORGANIC GLASS (METHYL METHACRYLATE)

A. V. Perelygin and N. V. Krylova

Department of Normal Human Anatomy (Head – Corresponding Member AMN SSR Prof. D. A. Zhdanov) of the I. M. Sechenov Order of Lenin First Moscow Medical Institute

(Presented by Active Member AMN SSSR V. V. Zakusov)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 126, January, 1960 .

Original article submitted April 28, 1959

Many valuable anatomical specimens are very brittle, which makes keeping them and working with them very difficult. The necessity for embedding such specimens (especially in the case of the small bones of the skull) has therefore arisen, using hermetically sealed glass jars or other forms of transparent media.

In recent years, attempts have been made to embed anatomical specimens in organic glass [1-8], etc.]. The reports so far published on this subject, however, have given only the general principles of the technique of the process, with no detailed description of the mode of embedding of the specimens; in some reports information is lacking on the composition of the plastic substance used. The incompleteness of these reports for practical use was the reason for the present investigation.

Under the guidance of D. A. Zhdanov, a method of embedding dry anatomical specimens in methyl methacrylate has been developed. This material is a plastic of the acrylic series – the methyl ester of methacrylic acid

As a result of a series of tests, we devised the following technique.

In order to free the methyl methacrylate from moisture and inhibitor, the monomer is distilled at first at 100° for 15 minutes, and then at 110° for 5 minutes. The monomer thus obtained contains a large quantity of moisture and cannot be used for subsequent polymerization. The methyl methacrylate used for embedding is obtained by further distillation at 130°, which temperature must be kept constant until the end of distillation.

In order to obtain a fore-polymer (condensed monomer), 0.75% by weight of benzoyl peroxide is added to the purified monomer as a catalyst, and the solution thus obtained is kept on a water bath at 60° for 1 hour; the temperature is then reduced to 40° and is maintained at this level for 2-3 hours. The fore-polymer thus obtained may be used for embedding.

Cockchafer, embedded in methyl methacrylate.

The specimen to be embedded in methylmethacrylate must be well dried. If separate bones are embedded, they must also be thoroughly dried and bleached. Hydrogen peroxide cannot be recommended for use in bleaching bones. The thoroughly cleaned, dry specimen is immersed in ready-prepared monomer and allowed to stand in a vacuum of 0.1-0.05 atmos; this ensures that the spaces in the specimen become filled with monomer. The specimen is then placed in a box, constructed of methyl methacrylate polymer plates, from 4 to 12 mm thick, and fixed by polished pegs, also made of polymer; after polymerization, the pegs cannot be seen – they become part of the block.

The embedding can then be proceeded with. Into the bottom of the polymer box, with the specimen mounted inside it, is gradually poured the fore-polymer in a quantity sufficient to ensure that the solution bathes the specimen from top to bottom and that no air bubbles were left behind on its surface. Afterwards, to protect surface of the block from dust and moisture, the box is covered on top with glass or parchment. The specimen thus embedded is kept in a dry room at 20-25° for 3-8 days (the time depends on the thickness of the object).

By the technique described, polymerization proceeds from below upward, and the end of the process can readily be observed by the movement of the border between polymer and monomer. In order to stabilize the process at the end of polymerization, the block is heated to 100-105° for 30 minutes.

The finished product is treated mechanically and polished by the usual method used with objects made from organic glass. The edges of the block must be trimmed evenly with a circular or carpenter's hand saw, and the surfaces cleaned, at first with a polishing stone or file, and later with a metal file or emery paper. The article is then well polished with paste, either on cotton polishing pads or by hand.

The method described can be used successfully for the preservation of collections of insects, butterflies, and so on (see figure). Preparations made in this way keep their natural size and color.

SUMMARY

It is a known fact that many anatomical preparations are very fragile, which makes it difficult to work with them and to keep them for a long time. A method for mounting dry anatomical preparations into organic glass (methylmethacrylate) has been developed.

A detailed description of the technique of mounting these preparations, particularly of small skull bones, is presented. This method may also be successfully employed to preserve collections of insects, butterflies, and other dry biological preparations. The preparations mounted by the above method retain their volume and natural color.

LITERATURE CITED

[1] V. A. Naumov, "Preparation of pathologoanatomical specimens in plexiglas". Veterinariya 10, 28-30 (1948).

[2] M. G. Prives, Methods of Preservation of Anatomical Specimens [in Russian] (Moscow, 1956).

[3] D. S. Sarkisov, "The use of plastics in histological technique." Arkh. patol. 1, 92-93 (1951).

[4] H. G. Goslar, Ein neues Verfahren zur Einbettung makroskopischer Preparate, Anat. Anzeig. 101, 5-9, 101-103 (1954).

[5] J. L. Kerus, An improved technique of embedding specimens in transparent plastic. Anat. Rec., 117, 345-352 (1953).

[6] M. R. Lyons and R. E. Johnson, Embedding stained mammary glands in plastic. Stain Technology 28, 4, 201-204 (1953).

[7] W. Peters, Die Einbettung biologischer Objekte in den Kunststoff Celodal unter dessen Emhullung mit einem Mantel aus Liepharz-P-Marken. Z. Wiss. Mikr. 62, 5, 327-333 (1955).

[8] G. G. Robertson and S. L. Moran, Plastic embedding of cleared human fetuses. Lab. Investig. Philadephia 23, 245-249 (1953).

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED

[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Ekspti. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriia	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Ekspti. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

PROCEEDINGS OF THE ALL-UNION SCIENTIFIC AND TECHNICAL CONFERENCE ON THE APPLICATION OF RADIOACTIVE ISOTOPES MOSCOW, 1957

Application of Radioactive Isotopes in Biochemistry and the Study of Animal Organisms
Jan.-Feb., 1959 heavy paper covers 20 papers, illustrated $50.00

Application of Radioactive Isotopes in the Food and Fishing Industries and in Agriculture
Jan.-Feb., 1959 heavy paper covers 16 papers, illustrated $30.00

Application of Radioactive Isotopes in Microbiology
Jan.-Feb., 1959 heavy paper covers 5 papers, illustrated $12.50

Radiobiology
Jan.-Feb., 1959 heavy paper covers 37 papers, illustrated $75.00

SPECIAL PRICE for the 4-VOLUME SET $125.00

Individual volumes may be purchased separately

The utilization of isotopes and radiation in biology, medicine, and agriculture is covered in 78 reports. Included in these significant papers are the latest Soviet techniques in the action of radiation on the living organism for the purpose of producing directed changes in plants and animals, curing of human illnesses and the utilization of isotopes as tagged atoms in the study of vital processes. Every biologist, chemist, health physicist, and physician employing the techniques should have access to this outstanding reference work.

Note: Individual reports from each volume are available at $12.50 each. We will gladly supply a detailed table of contents upon request.

CONSULTANTS BUREAU
227 WEST 17TH STREET, NEW YORK 11, N Y

THE LATEST SOVIET RESEARCH — IN TRANSLATION

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

Features research reports by leading Soviet authorities on *physiology, pathology, immunology, biophysics, pharmacology, oncology, morphology* and related fields. Translation is by special arrangement with the National Institutes of Health.

ANNUAL SUBSCRIPTION: 12 issues, **$20.00.**

BACK ISSUES: 1956-58 (12 issues per year), **$20.00** per year.

BIOCHEMISTRY

The most comprehensive Soviet periodical in this field. Each issue contains more than twenty papers on the biochemistry of plants and animals. Translation is by special arrangement with the National Institutes of Health.

ANNUAL SUBSCRIPTION: 6 issues, **$20.00.**

BACK ISSUES: 1956-58 (6 issues per year), **$20.00** per year.

PHARMACOLOGY AND TOXICOLOGY

This journal contains reports of the latest Soviet research on physiological effects of drugs, industrial hygiene, and related chemico-pharmacological-toxicological fields. The translation is published by special arrangement with the American pharmaceutical industry.

ANNUAL SUBSCRIPTION: 6 issues, **$25.00.**

BACK ISSUES: 1957-58 (6 issues per year), **$25.00** per year.

ANTIBIOTICS

This outstanding new publication surveys the entire field with timely research reports by leading Soviet scientists. It is translated and published with the financial assistance of a number of leading U.S. companies.

ANNUAL SUBSCRIPTION: 6 issues, **$25.00.**

Descriptive folders upon request

and—of special interest—

SOVIET RESEARCH IN PHARMACEUTICAL CHEMISTRY

Two invaluable collections which provide researchers with a convenient and inexpensive coverage of contemporary advances in Soviet pharmaceutical research.

Contents include pertinent articles which have appeared in chemistry journals translated cover-to-cover since 1949. Presents Western scientists with an excellent insight into the thinking, methodology and trends of their Soviet counterparts.

"a storehouse of factual references" —Journal of Chemical Education

Tables of contents and prices upon request.

CONSULTANTS BUREAU

227 West 17th Street, New York 11, N.Y.